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ELECTRICAL ENERGY OUTPUT OF THE RESTING STOMACH AS DETERMINED BY SHUNTING ITS POTENTIAL

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Although many investigators have suggested, partly on the basis of the results of experiments on non-living membranes and partly on the basis of potential measurements in living tissues, that electroendosmosis may be the mechanism by which osmotic work is performed by living organisms (1, 5, 6), the actual experimental evidence for this hypothesis is extremely meager. This hypothesis implies living tissues are capable of producing sufficient electrical energy for the performance of their osmotic work. A review of the literature reveals that there have been comparatively few studies on the production of electrical energy by living organisms. However, Stapp (11) has attempted to determine the ability of the frog skin to produce electrical energy by shunting its potential through a relatively low external resistance. He concluded that the electrical energy produced under the conditions of his experiments was in the neighborhood of 1 to 5 per cent of the total metabolic energy. Blinks (2) has reported the production through an external shunt of a continuous current of from 5 to 10 microâmperes from a single halycystis cell.

For an accurate determination of the amount of electrical energy produced by an organism the actual electrical circuits inside the tissue would have to be known. Since these internal circuits are not known it has to be assumed for the purposes of calculating the electrical energy that the circuit of the tissue consists of a potential and a resistance in series. Calculations based on this assumption would therefore represent only the minimum possible output of electrical energy (see discussion). Since the method of shunting the current through an external resistance is limited to determining the minimum possible output of electrical energy of a tissue, it would be interesting to know whether or not there is any depolarization of the potential upon the withdrawal of the current. In other words, to what extent is the ability of a tissue to maintain its potential taxed by the withdrawal of the maximum current. The present work is primarily concerned with this problem. It would also be of interest to determine by this method the electrical energy output of a tissue capable of doing considerable osmotic work. The dog's stomach was chosen because it is a

structure capable of producing considerable osmotic work in the secretion of HCl (4) and also because reports in the literature and preliminary experiments revealed that it possessed a relatively stable electrical potential which would be advantageous for the study of effects of current flow on the magnitude of the potential. Several workers have investigated the effect on the gastric potential of drugs that stimulated gastric secretion. Quigley et al. (9) report that these agents have no observable effect on the potential while Sarre (10) and Mislowitzer (7) report a decrease in the stomach potential after histamine stimulation. In preliminary experiments of the present investigation a decrease in the magnitude of the stomach potential was observed after histamine stimulation. Since the method of shunting cannot give information about the actual electrical energy expended inside a tissue, and since the magnitude of the potential was higher in the resting stomach than after histamine stimulation, it was decided to use the resting stomach as the material for this investigation.

METHODS. Dogs starved for 24 hours after preliminary ether anesthesia were injected with either barbital (200 to 250 mgm. per kgm.) or pernoston[1] (35 to 60 mgm. per kgm.). The stomach was exposed through an abdominal incision, ligatures were placed around the lower part of the esophagus and the pylorus, and an incision was made in the ventral wall of the stomach through which an electrode could be introduced. The stomach was washed out with large quantities of warm physiological saline. Under these conditions there was practically no spontaneous secretion, i.e., less than 1 cc. per hour.

An electrode introduced through the incision in the ventral wall was placed on the mucosal side on the ventral wall of the body of the stomach. Another similar electrode was placed on the serosal side directly opposite the inside electrode. An electrode consisted essentially of a circular shell of lucite containing a zinc plate which had previously been immersed in a saturated zinc amalgam, surrounded by saturated zinc acetate agar (2 per cent) with an outer layer of mammalian Ringer agar (2 per cent) to prevent zinc acetate from coming in contact with the stomach. Several other types of electrodes were tried but none were as satisfactory as the ones described above. Ag-AgCl electrodes showed considerable polarization with the current strengths of the same order of magnitude as those obtained from the stomach.

In order to prevent the electrodes from exerting undue pressure on the stomach one of the electrodes was fixed and the second electrode was free to swing. The support of the second electrode was placed so that the stomach supported a given fraction of the weight of this electrode. The actual pressure exerted by the electrode was less than 4 cm. of water.

These electrodes possessed a potential difference that was usually only a small fraction of a millivolt and showed very little polarization, i.e., usually less than $\frac{1}{2}$ millivolt change with a current density of $\frac{1}{2}$ milliampere per cm.^{2} for a period of five minutes. Several sizes of electrodes were used, all of relatively large surface area to minimize the contribution of neighboring areas, not directly under the electrodes, to the production of current.

[1] The Riedel-deHaen Co., New York, kindly supplied the pernoston used in these experiments.

In preliminary experiments with thin layers of Ringer agar a white deposit appeared on the stomach in a relatively short time. It was found necessary to use thicker layers of Ringer agar, i.e., about 1 cm. for each electrode. The thicker layers of Ringer agar increased the resistance of the electrodes, the average resistance of the electrodes (two electrodes in series) used in the experiments reported here being 16.4 ohms per 10 cm.^{2} of electrode area.

Leads from the electrodes were connected to a variable resistance box. The voltage drop across the resistance box was measured with either a potentiometer or a galvanometer. The resistance of the electrodes plus stomach was found to be relatively low and for the later experiments the galvanometer, calibrated as a voltmeter, was used in place of the potentiometer. The leads from the resistance box, for measuring the voltage drop across the box or directly from the electrodes, for open circuit voltage measurements, were connected to a resistance of approximately 26,000 ohms and the galvanometer leads were connected across portions of this resistance (100 ohms for open circuit voltage measurements) depending upon the sensitivity desired. With this arrangement wide variations in the resistance of the stomach and electrodes would have a negligible effect on the galvanometer deflection. The error in using this method for measurement of the open circuit voltage (referred to as potential in this paper) or the voltage drop across the resistance box was less than 0.2 per cent. Current flow through the resistance box was calculated from the determinations of the voltage drops. A direct current Wheatstone bridge was used for measuring the resistance of the electrodes. Since the potential difference of the electrodes was never actually zero the resistance was determined by measuring the apparent resistance in the two directions and averaging these values.

RESULTS. In the early experiments it was found that the potential of the stomach tended to decrease during the course of an experiment. However, in the later experiments in which precautions were taken to maintain the temperature of the stomach at a normal level, and in which a minimum of the anesthetic was used the potential was maintained at a relatively constant value for the duration of an experiment. The serosa was found to be positive in the external circuit to the mucosa in every experiment. The magnitude of the potentials was similar to those found by other investigators (7, 9, 12).

Effect of decreasing external resistance on current flow. In figure 1A, a typical experiment, the current produced by the stomach is plotted against the total external resistance of the circuit. The external resistance was regulated by varying the resistance of the variable resistance box, the total external resistance being equal to the resistance of the resistance box plus the resistance of the electrodes. The lowest external resistance was equal to the resistance of the electrodes plus 1 ohm. Over 40 similar experiments were performed and similar results were obtained in all of them. As is shown in the figure, decreasing the external resistance increases the current, the rate of increase being greatest when the resistance is less than 100 ohms.

Effect of current flow on the magnitude of the potential. An answer to the question as to whether there is depolarization of the potential upon the withdrawal of current may be obtained by applying the following well known principle of

physics.   If a potential difference exists between two points in a circuit, a potential and resistance in series can represent the circuit for predictions of the current in shunts connecting the two points.   That is, if there are two terminals on a closed box and nothing is known about the circuit inside the box, a resistance and potential of appropriate magnitudes arranged in series constitute an equivalent circuit for the actual circuit inside the box with respect to predictions of the amount of current flowing through shunts connecting the terminals of the box. This is true providing of course that the flow of current in itself does not cause a reduction in the magnitude of the potentials in the circuit.   Applying this principle to the stomach it follows from Ohm's law that

$$E = R_E I + R_S I \tag{1}$$

where E is the potential of the stomach, R_S the resistance of the stomach, R_E the resistance in the external circuit, and I the current in the external circuit. On rearranging

$$R_E I = E - R_S I \tag{2}$$

From equation 2 it can be seen that $R_E I$ vs. I can be represented by a straight line providing there is no decrease in E as the magnitude of the current increases. E is equal to the total IR drop in the circuit.   From the data presented above (R_E vs. I) $R_E I$ was calculated and plotted against I.   In figure 1A it can be seen that a straight line accurately represents this relationship.   This straight line relationship was found in all of the experiments except in a few early experiments in which measurements were attempted during periods in which the potential of the stomach was not very stable.   Inspection of equation 2 reveals that the slope of the line is numerically equal to the resistance of the stomach.   The resistance of the stomachs in these experiments is given in column R_S, table 1. The resistance of the stomach per square centimeter can be obtained by multiplying R_S by the electrode area.   The resistances were also determined by means of a direct current Wheatstone bridge.   The values obtained by the two methods were the same within the limits of error of the methods.   The measurements obtained by the Wheatstone bridge method are not given here because they are not essential to the thesis presented in this paper.

Effect of shunting for various periods of time on the current output.   In the above experiments the electrodes and stomach were connected to the variable resistance box just long enough to obtain a reading.   The time involved for a complete series of readings was about three minutes.   The question naturally arises as to whether the original output of current can be maintained over longer periods of time.   Figure 1B represents a typical experiment in which the current was shunted through the electrodes plus a 1 ohm resistor for a period of five minutes. It can be seen from the graph that apart from a small temporary initial decrease in the current, the current output remained at a relatively constant level.   Over 30 similar experiments were performed in which the current was shunted through the electrodes plus a 1 ohm resistor for periods of a few minutes to ½ hour.   In all of the experiments in which the open circuit voltage was relatively constant

before shunting (the majority of experiments), the current, during the period the shunt was applied, was maintained at approximately its initial value. It showed no tendency to decrease with time. In the experiments in which the open circuit voltage was either gradually decreasing or increasing, the voltage drop through the 1 ohm resistor showed the same proportionate tendency to change as did the open circuit voltage before shunting. Interpolation of the open circuit
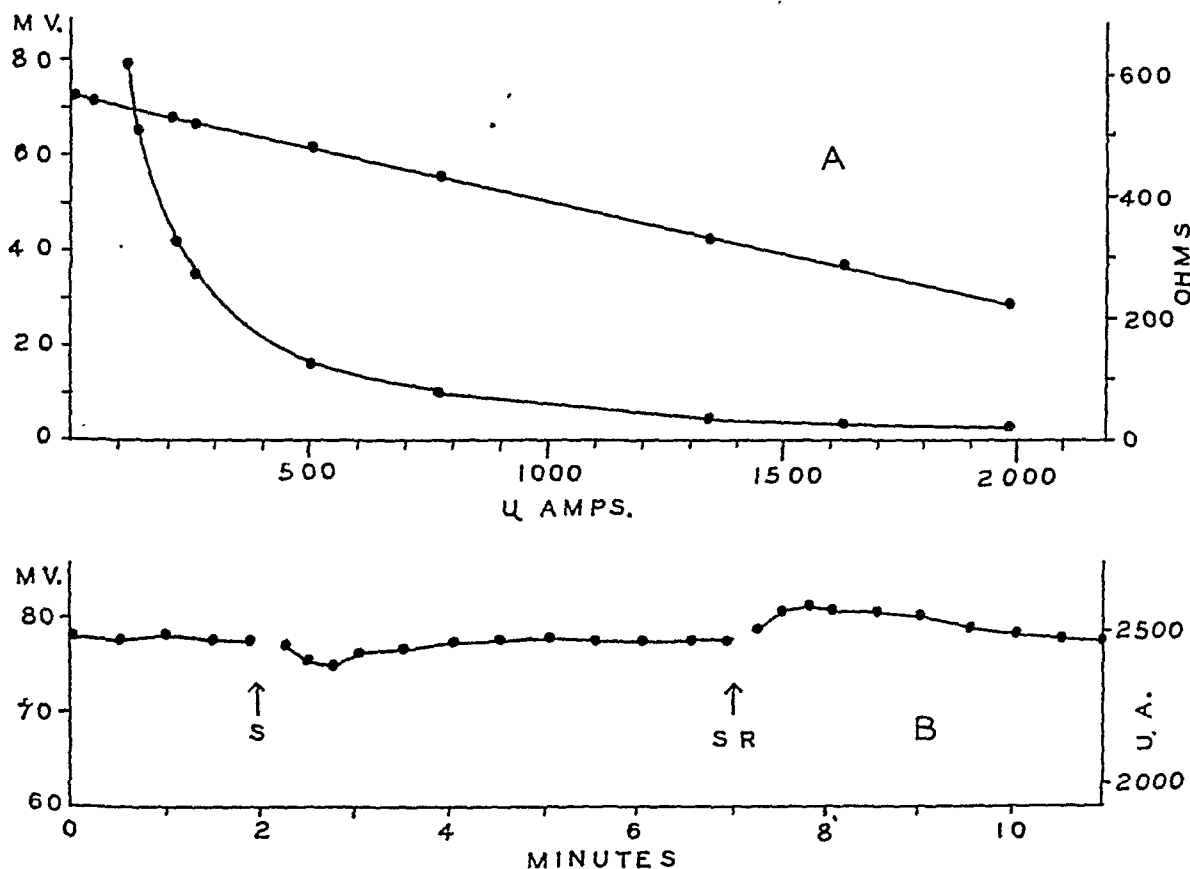
Fig. 1A. The curved line represents the current in the external circuit in microamperes vs. resistance of the external circuit (resistance of the electrodes plus resistance of variable resistance box) in ohms. The lowest external resistance in this experiment was 16.5 ohms (resistance of the electrodes, 15.5 ohms plus 1 ohm).

The straight line represents the IR drop in the external circuit in millivolts (R_EI) vs. the current in the external circuit. Dog 3 of table 1.

Fig. 1B. Effect of shunting the stomach through the electrodes plus a 1 ohm resistor. The line before S and after SR represents the open circuit voltage of the stomach in millivolts (left-hand scale). At S the stomach was shunted; at SR the shunt was removed. The line between S and SR represents the current in the external circuit in microamperes (right-hand scale) during the period in which the shunt was applied. Dog 10 of table 1.

voltages before and after shunting indicated that shunting did not observably influence the rate of change of the potential.

It can be seen in figure 1B that after the shunt was removed there was a small temporary increase in the potential. The two effects, the small temporary decrease in the current after applying the shunt and the small temporary increase in the potential after removal of the shunt, were observed in the majority of

the experiments in which the magnitude of the current exceeded 150 microamperes per square cm. It was never more than 3 per cent of the average values of the current or potential. Controls demonstrated that these effects could not be due to peculiarities of the galvanometer used for measuring the potentials. Current from a comparable potential and resistance when connected to the galvanometer showed only a difference of $\frac{1}{4}$ mv. between the initial 15 second reading and the subsequent readings. Controls in which comparable currents were passed through the electrodes showed a maximum change in P.D. of less than $\frac{1}{2}$ mv. These effects seem therefore to be mainly dependent on the activity of the stomach.

TABLE 1

	DOG									
	1	2	3	4	5	6	7	8	9	10
R_S	11.1	7.5	21.0	12.1	24.5	20.0	24.4	11.2	10.2	12.1
Elec. area	13.8	21.3	13.8	11.6	11.6	11.6	11.6	10.7	10.7	10.7
I_{max}	938	3,040	2,040	1,210	1.520	1.170	1,340	2,950	1,830	2,580
$\frac{I_{max}}{cm.^2}$	68	142	150	104	131	101	115	276	171	241
E	27.6	47.1	71.5	29.1	55.5	41.2	51.5	80.5	44.4	80.8
$\frac{EI}{cm.^2}$	1.88	6.74	10.7	3.0	7.3	4.2	5.9	22.2	7.6	19.4

R_S is the resistance of the stomach in ohms as determined from equation 2. "Elec. area" is the area of electrodes in square centimeters. I_{max}. is the current in microamperes obtained when external resistances equal the resistance of the electrodes plus 1 ohm. $\frac{I_{max}}{cm.^2}$ is the current in microamperes I_{max}. divided by the electrode area. E is the average of the potentials in millivolts before and after I_{max}. was obtained. $\frac{EI}{cm.^2}$ is the rate of electrical energy production in microwatts per square centimeter obtained by multiplying E by $\frac{I_{max}}{cm.^2}$. Pernoston was used in the third and last four experiments; barbital was used in the other experiments.

The maximum current obtained for each of the dogs is given in column I_{max}. of table 1. The area of the electrode used in each of the experiments is also given in table 1. The maximum current per square centimeter was calculated by dividing the maximum current by the electrode area.

Calculation of electrical energy output of the stomach. Since current can be drawn from the stomach over comparatively long periods of time and since there is no maintained depolarization of the potential during the flow of current, the rate of electrical energy production under these conditions can be calculated by multiplying the current by the open circuit voltage. In table 1 column $\frac{EI}{cm.^2}$ gives the rate of production of electrical energy in microwatts per sq. cm. This was calculated from the average of the open circuit voltage before and after shunting

(column E) and the maximum current output (column $\frac{I_{max.}}{cm.^2}$) per cm.^{2}. The average value for electrical energy output was 8.9 microwatts per sq. cm.

DISCUSSION AND CONCLUSIONS. The foregoing experiments demonstrate that as much as 67.8 to 276 microamperes per cm.^{2} can be produced continuously by the resting stomach without any appreciable maintained depolarization of the potential. Had there been marked depolarization of the potential it would indicate that these amounts of current would be near the maximum that the potential was capable of producing. The fact that there was no appreciable depolarization suggests that the gastric potential is inherently capable of delivering currents of greater magnitudes than those actually obtained. It is to be recalled that the calculations of the electrical energy produced by the stomach were made on the assumption that a potential and resistance represented the actual circuit. It is obvious that if any other circuit better approximated the actual circuit of the stomach, the amount of electrical energy produced would be greater than that calculated. For example, if there were a shunt across the potential inside the wall of the stomach and the resistance of the shunt was small compared to the resistance of the tissues from the locus of the potential to the serosal or mucosal surfaces then the amount of electrical energy might be many times greater than that calculated. It should be pointed out here with respect to the problem of determining the actual circuit of the stomach that, since a resistance and a potential in series constitute an equivalent circuit for the stomach, the sort of data presented in this paper will not enable one to determine the actual circuit of a tissue. A resistance and potential is an equivalent circuit for the data presented in this paper and it is not to be assumed that it is an equivalent circuit for all of the electrical characteristics of the stomach.

Inspection of figure 1A in which current is plotted against resistance might suggest that as the external resistance approaches zero the current increases to infinity and therefore the problem of determining the ability of the stomach to produce electrical energy is primarily one of obtaining lower values of the external resistance. That this is not the case can be shown by an examination of the equation representing the above relationship, i.e.,

$$I = \frac{E}{R_E + R_S} \tag{3}$$

obtained from equation (1). It is obvious that as R_E approaches zero I approaches $\frac{E}{R_S}$.

In order to obtain an approximate idea of the relative order of magnitude of the electrical energy output a comparison of these values with the values found in the literature for the amount of metabolic energy produced by the resting stomach would be pertinent. Ni and Lim (8) give 0.004 cc. of O_2 per gram per minute as an average value of the O_2 consumption of the resting vivi-perfused stomach. Boenheim (3) gives 166 mm.^{3} of O_2 per 100 grams per minute. For purposes of comparison the metabolic energy was calculated in electrical units.

The above figures in microwatts per sq. cm. using Ni and Lim's data for conversion from grams to sq. cm. are 472 and 200 respectively. Using the average value for the electrical energy obtained in these experiments, 8.9 microwatts per sq. cm. (average of the values in column $\frac{E_1}{cm.^2}$ of table 1), the per cent of total metabolic energy obtained as electrical energy is 1.9 per cent and 4.4 per cent, which is approximately the same percentage that Stapp (11) found for the frog skin. The actual amount of electrical energy production found by Stapp for the frog skin was about one-tenth of that found for the stomach.

The question then arises: Is there enough electrical energy produced by the stomach to account for the osmotic work performed in the secretion of gastric juice? Calculations reveal that the amount of electrical energy per stomach per 24 hours, using the maximum rate of electrical energy production (22.2 microwatts per sq. cm.), is equivalent to the minimum free energy necessary to produce approximately 200 ml. of gastric juice (using Davenport's (4) value of 820 cal. per liter). Although the stomach is capable of producing more gastric juice than the above amount, it is nevertheless of the same order of magnitude as the amounts actually produced. The fact that there was no maintained depolarization of the potential during the flow of the maximum current suggests that the stomach potential is inherently capable of producing sufficient energy for the secretion of gastric juice.

SUMMARY

The potential of the "resting" body of the dog's stomach was shunted through electrodes which had a resistance of approximately the same order of magnitude as the stomach. Currents of 67.8 to 276 microamperes per square centimeter could be drawn off continuously. Evidence is presented that there is no maintained depolarization of the potential upon the withdrawal of currents of the above magnitudes.

REFERENCES

(1) BEUTNER, R. Physical chemistry of living tissues and life processes, p. 62-66. Williams & Wilkins Co., Baltimore, 1933.
(2) BLINKS, L. R. Symp. on Quant. Biol. 1: 127, 1933.
(3) BOENHEIM, F. Ztsch. f. d. ges. exper. med. 71: 108, 1930.
(4) DAVENPORT, H. W. AND R. B. FISHER. This Journal 131: 165, 1940.
(5) JACOBS, M. H. Cowdry's General cytology, p. 132-133. Univ. of Chicago Press, 1924.
(6) KELLER, R. Biochem. Ztschr. 214: 395, 1929.
(7) MISLOWITZER, E. AND S. SILVER. Biochem. Ztschr. 256: 432, 1932.
(8) NI, T. G. AND R. K. S. LIM. Chinese J. Physiol. 2: 45, 1928.
(9) QUIGLEY, J. P., J. BARCROFT, G. S. ADAIR AND E. N. GOODMAN. This Journal 119: 763, 1937.
(10) SARRE, H. Ztschr. f. Biol. 95: 135, 1934.
(11) STAPP, P. Proc. Soc. Exper. Biol. and Med. 46: 382, 1941.
(12) SWYNGEDAUW, J. Compt. rend. Soc. biol. 98: 1431, 1928.

A COMPARATIVE STUDY OF THE OXYGEN CONSUMPTION OF THE VERTEBRATE RETINA, WITH ESPECIAL REFERENCE TO THE NUCLEO-PROTOPLASMIC RATIO

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The retina affords a number of unusual opportunities for studies on the respiratory activity of nervous tissue. Numerous contributions have been made to the literature on the gross metabolism of this tissue. Among the more recent studies are those of Adler (1935), Kodama (1936), Krebs (1929), Kubowitz (1929), Lindeman (1940), and Jongbloed and Noyons (1936).

In many respects the respiratory activity of the retina resembles the gray matter of the brain. But the retina, as a tissue, affords definite advantages over brain tissue for the metabolic studies of nerve cells. First, it probably contains a greater concentration of neural elements per unit mass than any other region in the nervous system. Secondly, it is thin enough to fall within the thickness generally required for respiratory studies of tissue in vitro, thus eliminating the necessity of slicing.

This study deals largely with the experimental analysis of the respiratory activity of nerve cells in the retina of several vertebrates. An attempt is made to secure additional information about the relative rates of nuclear and cytoplasmic respiration in neural tissue. These data provide a basis for computing the relative rates of oxygen consumption of individual nerve cells and their nuclei in the retina.

MATERIAL. The following animals, representatives of three classes of vertebrates, were used in this study:[1] Sucker (Catostomus catostomus), shiner (Notemigonus crysoleucas), chub (Leucosomus corporalis), frog (Rana pipiens), toad (Bufo americanus), necturus (Necturus maculosus), tortoise (Clemmys muhlenbergii), garter snake (Thamnophis sirtalis), and alligator (Alligator mississippiensis). All the specimens, except the tortoise and alligator, were collected locally during the spring and summer months and were used experimentally within two weeks of their capture. In each case the tissue used was the retina.

METHODS. The retina was prepared for experimental purposes as follows: The animal was decapitated and the eyes removed. The eye was placed in Ringer's solution and the cornea dissected away. The lens was then removed, and with the aid of a curved forceps the retina lifted intact from its position in the eye.

The oxygen consumption was measured by the direct method of Warburg in air and at 25.3 degrees centigrade. Vessels with a capacity of approximately 5 milliliters were employed on the manometers. All measurements were made in 1 ml. of Ringer's solution, buffered with phosphates to a pH of 7.5. The Ringer's

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[1] Jordan, D. S. Manual of the vertebrate animals. 13th ed.

solution was prepared each time from stock solutions of pure m/9 NaCl, m/9 KCl, and m/9 CaCl$_2$, in proportions of 100:1.5:1, respectively. Forty per cent KOH was used in the central well to absorb the carbon dioxide. The rate of oxygen consumption was computed from an average of the first 2 hours, and expressed in cubic millimeters oxygen consumed per milligram wet weight of tissue per hour.

The technique employed for counting and measuring the nuclei was similar to that used by Pearce and Gerard (1942) for frog brain tissue. In brief the procedure was as follows: After removing the retinae from the respiratory chambers they were carefully blotted and weighed. They were then transferred to small test-tubes containing exactly 2 ml. of 25 per cent acetic acid in water. Gentle shaking reduced the tissue to a fine suspension of intact nuclei and dissolved cytoplasm. One-fourth milliliter of 1 per cent methylgreen was added to stain the nuclei. Approximately 15 minutes was allowed for maximal staining. Samples were removed from the test-tube with a white cell diluting pipette and a drop of the suspension was placed under the cover glass of a hemocytometer counting chamber. The nuclei in 25 large squares were counted for each sample and at least 3 samples from each suspension were averaged. The variation between different samples of a suspension was seldom more than five per cent. The number of nuclei per milligram of retina was calculated for each suspension.

The nuclear dimensions were measured with an ocular micrometer at a magnification of approximately 450 ×. The long and short diameter of 1000 nuclei was measured for each representative of vertebrate retina studied.

The protoplasmic volume of the whole retina was measured by a method employing Archimedes Principle. The procedure consisted first, of weighing the retina in air, while suspended from a fine strand of silk thread which had been carefully oiled to reduce surface tension. The retina was then weighed suspended in a dish containing m/9 NaCl. The weight of the thread was subtracted from both weights. The volume was computed by using the formula

$$\text{Volume} = \frac{W_A - W_{\text{NaCl}}}{d_{\text{NaCl}} \times W_A} = \text{cubic millimeter/milligram of tissue}$$

W_A Weight in air.
W_{NaCl} Weight in NaCl.
d_{NaCl} Density of the NaCl solution.

It will be noted that connective and non-neural tissues are not taken into consideration when computing the values for neural elements. These corrections are difficult to determine and at best can only be estimated. When the relative number of nerve cells per unit mass of retina is compared with an equal mass of brain tissue, the retina greatly exceeds the average brain sample in cell population. This is also true for total nuclear mass per milligram of tissue. If it can be assumed that the ratio of nucleus to cytoplasm is approximately the same in the cells of the retina as in brain tissue, the greater number of neural units in the retina would considerably reduce the non-neural elements. Pearce and Gerard (1942) found that the average brain sample contained approximately 10 per cent endothelial cells, with the anterior olfactory

nucleus having as low as 6.5 per cent.   In view of the greater nerve cell population in the retina it seems reasonable to assume that the amount of non-neural tissue would be somewhat lower than that in the average brain sample.   Moreover, when one considers that the non-neural cells in the average frog brain sample is estimated to consume only about 0.1 per cent of the total oxygen uptake per hour (Pearce and Gerard, 1942), it logically can be considered quite unlikely that the non-neural elements would contribute any more to the total metabolism of the retina.   This, coupled with the possibility of various technical errors involved in cytological measurements, would no doubt cancel out the non-neural tissue as a value to be considered in the computations.

RESULTS AND DISCUSSION.   .It seemed desirable, as the first step in the experimental part of this study, to determine the relative number of neural cells in the retina of the different vertebrates being studied.   By counting the number of nuclei in a measured volume of tissue suspension a fairly accurate determination

TABLE 1

Nuclear count per milligram of retina $(\times 10^3)$

ANIMAL	EXPERIMENTS	MEAN NUMBER OF NUCLEI	STANDARD DEVIATION	PROB. ERROR OF MEAN
Sucker................	9	592	2.8	0.61
Shiner................	10	925	14	3.04
Chub.................	8	836	31	7.4
Necturus.............	8	72.9	6	1.4
Frog.................	10	325	5.2	1.13
Toad.................	9	419	14	3.1
Tortoise.............	8	287	22	5.3
Snake................	9	708	36	8.09
Alligator............	8	743	50	11.7

of the cells was obtained.   The term "nerve cell," as it is frequently used, refers to all neural cells irrespective of their modification or function.

The data contained in table 1 form a summary of the nuclear count for the various vertebrate retinae studied.   The mean number of nuclei represent an average of at least 25 counts on from 8 to 10 retinae.

The group as a whole displayed a rather wide range of variation in the number of nerve cells per unit mass.   It should be noted that the one extreme is represented by the necturus with a count of 72,000 cells and the other extreme by the shiner with 925,000.   Between these two extremes there is a fair representative distribution.

The next step in the cytological study involved the measurement of nuclear volumes.   Assuming that the nucleus be either an ellipsoid or a sphere, the volume can be computed from two measured diameters.   Where one diameter is greater than the other, it is assumed to be an ellipsoid and the volume can be computed from the formula, $V = \frac{\pi}{6} d^2 1$.   Where the two diameters are equal, the mass is assumed to be a sphere and the volume is computed from the formula $V = \frac{\pi}{6} d^3$.

Measurements of the long and short diameters were made for 1000 nuclei from several suspensions of each vertebrate retina used in this study. Those nuclei having the same dimensions were then classified into groups and their individual as well as their total volumes computed. The total nuclear volume per milligram of retina was obtained by multiplying the mean nuclear volume per nucleus, computed on 1000 nuclei, by the mean number of nuclei per milligram of retina.

The measurements of total protoplasmic volume per milligram of retina were made upon the retina of the frog, toad, sucker, and tortoise. The values obtained in each case were all so nearly the same, within the limits of experimental error, that a mean was obtained from 14 determinations on retinae from the above four animals. The mean protoplasmic volume of the whole retina, as determined in this manner was 0.9746 ± 0.0018 cu. mm. per milligram of tissue. This value was used to compute the nucleo-protoplasmic ratio of all retinae. By dividing the total nuclear volume by the total protoplasmic volume a ratio is obtained. A summary of these data is found on table 2.

TABLE 2

A comparison of the nuclear and protoplasmic volumes

	SUCKER	CHUB	SHINER	NEC-TURUS	FROG	TOAD	TOR-TOISE	SNAKE	ALLI-GATOR
	Nuc./mgm./retina. $\times 10^3$								
	592	836	925	72.9	325	419	287	708	743
Mean nuc. vol./mm.^{3} $\times 10^{-7}$..........	1.51	1.05	0.86	40.1	2.78	2.59	2.37	1.18	2.19
Total nuc. vol./mg. /ret.mm.^{3}........	0.089	0.088	0.0795	0.292	0.106	0.108	0.068	0.083	0.175
Nucleo-protoplasmic ratio........	0.093	0.093	0.082	0.300	0.109	0.111	0.070	0.088	0.180

As one would expect, there is a reasonably close relationship between the mean nuclear volume and the number of cells per milligram of tissue. When the total nuclear volume is compared with the total protoplasmic volume a few cases stand out as not conforming to the general pattern. Particular attention is directed to the necturus, alligator and the tortoise. The nucleo-protoplasmic ratio is 0.300 for the necturus, 0.180 for alligator, and 0.070 for the tortoise, as compared to an approximate ratio of 0.09 for the other retinae studied.

These findings suggest a possible approach to the question: How much does the nucleus as a mass contribute to the total oxygen consumption of the cell? If the assumption is made that the whole protoplasmic mass contributes equally to the total respiration of the cell, as claimed by Pearce and Gerard (1942), for frog brain neurones, then the ratio of nucleus to cytoplasm within a cell should not be a factor in determining its rate of metabolism. In other words, a cell with a nucleo-cytoplasmic ratio of 0.300 might have a rate of oxygen consumption of the same magnitude as one with a ratio of 0.07. On the other hand, if the nucleus and cytoplasm have appreciably different rates of metabolism the ratio of one to the other might conceivably influence the rate of the whole cell.

In order to test this assumption, oxygen consumption measurements were made on various vertebrate retinae. The data contained in table 3 summarize the results of 82 experiments on 9 different vertebrates.

It is obvious that there is little justification for comparing the mean respiratory rates of the different retinae. However, it is interesting to compare the cell populations, nuclear volumes, and nucleo-protoplasmic ratios of the various retinae with the amount of oxygen consumed by each. Such an examination reveals that there is no correlation between the number of cells and the rate of oxygen consumption. There is no more similarity in the rate of respiration between retinae having approximately the same number of cells than there is between retinae which differ greatly in cell count. This lack of correlation also exists when respiratory rates are compared to nuclear volumes and nucleo-protoplasmic ratios. It will be noted that the mean oxygen consumption of retinae with similar nucleo-protoplasmic ratios are no more comparable than those with widely different ratios. A comparison of several specific cases will illustrate this

TABLE 3

Mean oxygen consumption of vertebrate retina mm³ per mgm. per hour

ANIMAL	EXPTS.	MEAN O₂ CONSUMED	STANDARD DEVIATION	PROB. ERROR OF MEAN
Sucker................	10	0.592	0.063	0.012
Shiner................	9	0.768	0.028	0.006
Chub.................	9	0.756	0.010	0.002
Necturus.............	9	0.664	0.035	0.008
Frog.................	10	0.467	0.027	0.005
Toad.................	8	0.540	0.090	0.022
Tortoise.............	9	0.445	0.035	0.008
Snake................	10	0.569	0.035	0.006
Alligator............	8	0.529	0.033	0.008

point. The necturus retina with a ratio of 0.300 and a mean rate of oxygen consumption of 0.664 cu. mm. per milligram per hour when compared with several retinae having a ratio of approximately 0.09, has a respiratory rate higher than some and lower than others. This also holds true for the alligator which has a ratio of 0.180. Moreover, the oxygen consumptions of retinae with similar nucleo-protoplasmic ratios are no more consistent.

If a sufficient sampling has been made and one is justified in making the above comparisons, this evidence would seem to indicate that the whole protoplasmic mass contributes equally to the total metabolism of the retinal nerve cells. These findings are also in agreement with the findings of Pearce and Gerard (1941) for frog brain cells. If this is a reasonable assumption it should be possible to compute the oxygen consumption of the individual nuclei and in turn, the whole cell. It is a rather simple matter to compute the mean rate of respiration per cell. This can be done by simply dividing the oxygen consumed per milligram of retina by the number of nuclei per milligram of tissue. The mean oxygen consumption per cell is of little significance since it merely represents a value obtained upon a hypothetical cell. However, by employing this value in

conjunction with measurements of nuclear and protoplasmic volumes (table 2) the mean oxygen consumption of a cell of known volume can be approximated.

Before the oxygen consumption of the individual cells could be evaluated it was necessary to know their volumes. The method employed in obtaining these data postulates a nucleo-cytoplasmic ratio for each cell on the basis of values obtained for the nucleo-protoplasmic ratio per milligram of retina; hence the values for cell volumes are only relative and can only be considered as approximations. The author recognizes the limitations of this method, but because of the practical difficulty of measuring accurately the volume of a single nerve cell this procedure appeared to be a plausible solution to the problem.

TABLE 4

A comparison of the mean rates of oxygen consumption of cells and nuclei

	SUCKER	CHUB	SHINER	NEC-TURUS	FROG	TOAD	TOR-TOISE	SNAKE	ALLIG.
	Mean O_2 consumed per mgm./ret./ per hr. mm.^{3}								
	0.592	0.768	0.756	0.664	0.467	0.540	0.445	0.569	0.529
Mean O_2 consumed per cell/hr. mm.^{3} $\times 10^{-6*}$..........	1.0	0.90	0.80	9.11	1.44	1.29	1.55	0.80	0.71
O_2 consumed by nuc. per mgm. ret. per hr. mm.^{3}†....	0.054	0.066	0.065	1.98	0.092	0.0603	0.031	0.0475	0.0296
Mean O_2 consumed per nucleus. mm.^{3} per hr. $\times 10^{-7}$‡..	0.94	0.77	0.71	25.7	3.8	1.44	1.08	0.67	1.24

* $\dfrac{O_2 \text{ consumed per mgm. per hr.}}{\text{Nuclear count per mgm. retina.}}$

† $\dfrac{O_2 \text{ consumed per mgm. per hr.} \times \text{total nuclear volume per mgm. retina}}{\text{Protoplasmic volume per mgm. of retina.}}$

‡ $\dfrac{\text{Oxygen consumption of nuclear mass per mgm. retina}}{\text{Nuclear count per mgm. retina.}}$

By utilizing the data in tables 2 and 4 the oxygen consumption of nuclei of different volumes was computed. This was done according to the following formula:

$$\frac{\text{Oxygen consumption of mean nuclear volume} \times \text{volume of nucleus}}{\text{Mean volume of nuclei}}$$

The formula for computing the individual cell volumes employed the following relationships:

$$\frac{\text{Protoplasmic volume per mgm. of retina} \times \text{volume of nucleus}}{\text{Total nuclear volume per mgm. of retina}}$$

The oxygen consumption of the cells with different volumes was computed as follows:

$$\frac{\text{Oxygen consumption of the nucleus} \times \text{volume of the cell.}}{\text{Volume of the nucleus.}}$$

Table 5 is an analysis of the cellular respiration of 3 vertebrate retinae, one representative from each class. Columns 1 and 2 give the nuclear and cell volumes respectively, 3 and 4 the corresponding rate of oxygen consumption for the nucleus and cell, and 5, a percentage grouping of nuclei according to volume.

TABLE 5

The relative oxygen consumption of nuclei and cells of different volumes

| NECTURUS | | | | | SHINER | | | | | ALLIGATOR | | | | |
| Volume (mm.³) | | O₂ consumed (mm.³) | | No. nuc. | Volume (mm.³) | | O₂ consumed (mm.³) | | No. nuc. | Volume (mm.³) | | O₂ consumed (mm.³) | | No. nuc. |
Nuc. 10⁻⁷	Cell 10⁻⁷	Nuc. 10⁻⁷	Cell 10⁻⁷		Nuc. 10⁻⁷	Cell 10⁻⁷	Nuc. 10⁻⁷	Cell 10⁻⁷		Nuc. 10⁻⁷	Cell 10⁻⁷	Nuc. 10⁻⁷	Cell 10⁻⁷	
				per cent					*per cent*					*per cent*
16.9	53.7	10.8	35.6	7	0.265	3.17	0.160	1.93	52	0.79	4.41	0.44	2.45	3
21.6	71.3	11.3	47.7	11	0.391	4.70	0.238	2.88	8	0.87	4.85	0.49	2.73	37
23.4	77.2	14.8	48.8	8	0.530	6.35	0.323	3.90	2	1.17	6.28	0.66	3.68	1
35.3	116.0	22.2	73.0	5	0.870	9.85	0.720	8.17	20	1.75	9.38	0.99	5.50	20
39.1	129.0	25.0	83.0	38	1.170	14.0	0.835	10.0	3	2.37	13.20	1.33	7.43	23
					2.370	28.5	1.48	17.8	9	2.84	15.80	1.60	8.82	4
46.6	164.0	29.7	101.0	8	3.230	38.8	1.97	23.8	4	3.23	18.00	2.00	11.10	4
53.6	177.0	34.0	112.0	4	6.960	83.5	4.25	51.1	2	4.41	24.40	2.48	13.80	2
55.7	183.5	35.4	116.0	6						6.96	38.80	3.92	21.80	5
66.2	218.5	44.3	138.0	9						8.97	49.90	5.05	28.00	1
83.0	274.0	53.0	175.0	1										
91.9	303.0	81.8	246.0											

SUMMARY

1. Cytological measurements and oxygen consumption determinations were made upon the following vertebrate retinae: Sucker, Chub, Shiner, Frog, Necturus, Toad, Snake, Tortoise, and Alligator.

2. The cell population of the different retinae was found to range from 72,900 per milligram in the necturus, to 925,000 per milligram in the shiner, with a good range of distribution between these extremes.

3. The ratio of nucleus to protoplasm was approximately 1:10 in all cases except the necturus, alligator and tortoise, which had ratios of 1:3.3, 1:5.56, 1:14.3, respectively.

4. No apparent correlation seemed to exist between the nucleo-protoplasmic ratios and the mean rate of oxygen consumption of the various retinae. This was interpreted to indicate that the whole protoplasmic mass contributed equally to the total metabolism of the cells.

5. The oxygen consumption of the individual nerve cells was computed in relation to their nuclear and cell volumes.

REFERENCES

(1) ADLER, F.   Arch. Ophth. **6**: 901, 1931.
(2) KUBOWITZ, F.   Biochem. Ztschr. **204**: 475, 1929.
(3) LINDEMAN, V.   Physiol. Zool. **13**: 411, 1940.
(4) KREBS, H.   Biochem. Ztschr. **189**: 57, 1927.
(5) KODAMA, S.   Tohoku J. Exper. Med. **28**: 423, 1936.
(6) PEARCE, J. AND R. W. GERARD.   This Journal **136**: 49, 1942.
(7) JONGBLOED, J. AND A. K. NOYONS.   Ztschr. f. Biol. **97**: 399, 1936.

THE EFFECT OF DIETHYLSTILBESTROL ON THE BLOOD PRESSURE OF NORMAL AND HYPOPHYSECTOMIZED RATS

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A lowering of the blood pressure following the removal of the hypophysis has been reported for a number of animals. Wyman and tum Suden (1) measured the blood pressure of the hypophysectomized rat by carotid cannula and found it to be subnormal. Recently Williams, Harrison and Grollman (2) have described a method by which repeated systolic blood pressures can be obtained on the unanesthetized rat and it was a purpose of this investigation to use this method and record any blood pressure changes following hypophysectomy.

Estradiol benzoate and diethylstilbestrol have been shown to elevate the blood pressure of the normal rat, in some cases, to hypertensive levels (3). In view of this action of stilbestrol in the normal rat it appeared of interest to compare its action on the blood pressure of the normal and hypophysectomized rat.

MATERIALS AND METHODS. Forty-nine male rats, weighing 250 to 340 grams and ranging from 100 to 176 days in age, were used for these experiments. Of this group, 29 were hypophysectomized. The systolic blood pressure was determined by the method of Williams, Harrison and Grollman (2). Several pressure determinations were made before hormone administration or hypophysectomy, because the first two or three determinations were usually found to be higher than normal. However, the animals readily became habituated to the apparatus and normal rat pressures exhibited little variation.

Diethylstilbestrol[2] was dissolved in sesame oil and administrated intramuscularly in daily dosages of 1 mgm. contained in 0.05 cc. of oil.

Pituitary capsules were serially sectioned, stained with Mallory's connective tissue stain and examined microscopically. Data from only completely hypophysectomized rats are presented.

RESULTS. *Normal rats.* The systolic blood pressure of normal rats fluctuated between 111 and 118 mm. Hg as an average for eleven animals over a twenty-nine day test period (fig. 1).

Injection of 1 mgm. of stilbestrol daily into nine normal rats caused a gradual rise in systolic blood pressure in eight animals. After ten injections a definite upward trend of the pressure was established and after twenty-eight days of hormone administration the average pressure of the stilbestrol treated rats was twenty millimeters above the normal. The degree of rise varied from 0 to 38 mm. in different rats. Individual cases exhibited four instances in which the

pressure ranged between 145 and 153 mm., the latter being the highest pressure recorded (fig. 1).

Hypophysectomized rats. Following hypophysectomy the blood pressure dropped rapidly, being 76 mm. for an average of nine rats following a postoperative interval of eight days. At about this time the blood pressure became stabilized and maintained approximately an 80 millimeter average for the twenty-nine day investigational period. Individual systolic pressures following hypophysectomy ranged from 65 to 90 mm. and represented an average decrease in pressure of 30 to 35 mm. (fig. 2, curve *A*). We have also determined the blood pressure of nine rats following a post-operative period of one hundred eighty to
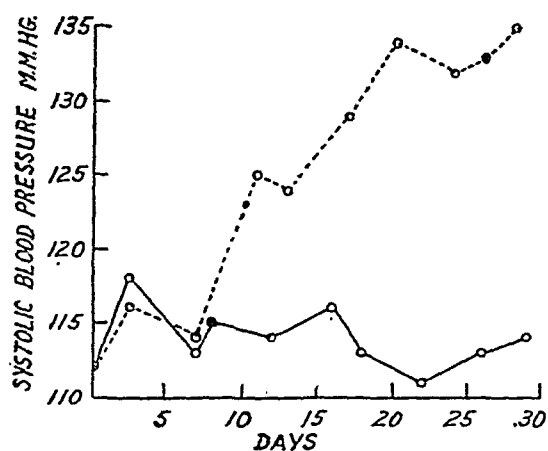
Fig. 1
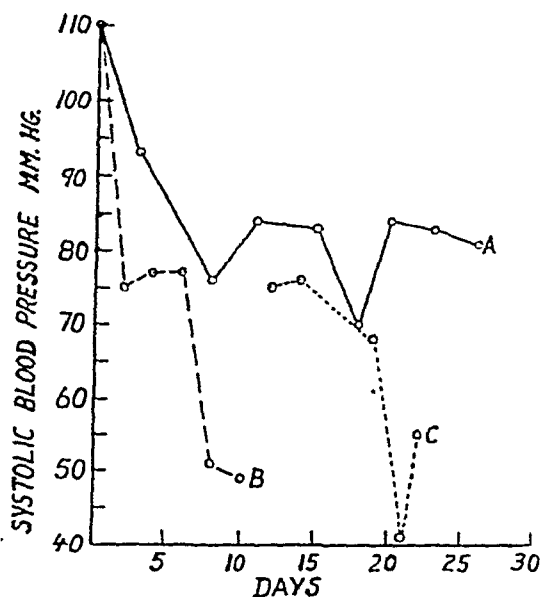
Fig. 2

Fig. 1. Effect of diethylstilbestrol on systolic blood pressure of normal rats. ○——○ normal untreated rats; ○----○, normal rats injected with 1 mgm. of stilbestrol per day.

Fig. 2. Effect of diethylstilbestrol on the systolic blood pressure of hypophysectomized rats. Curve *A*, change in systolic blood pressure of hypophysectomized rats. Curve *B*, systolic blood pressure of hypophysectomized rats injected with 1 mgm. of stilbestrol starting the day of hypophysectomy. Curve *C*, systolic blood pressure of hypophysectomized rats injected with 1 mgm. of stilbestrol daily starting 12 days postoperatively.

two hundred twenty-two days. Several determinations were made on each rat during the course of a week and blood pressures were found to range from 71 to 83 mm.

Starting the day of hypophysectomy, seven rats were injected with 1 mgm. of stilbestrol daily for ten days. The blood pressure decline was markedly accentuated, reaching 75 mm. in three days. At the end of ten days the average blood pressure was 50 mm., which represented a pressure of 25 mm. below that of untreated hypophysectomized rats (fig. 2, curve *B*). One animal failed to survive the ten-day injection period but this appeared to approximate the maximum treatment period the rat could tolerate.

To offset the combined effect of stilbestrol and hypophysectomy, the injection of stilbestrol was withheld until twelve days after the operation at which time the blood pressure is essentially stabilized. Injection of stilbestrol in 1 mgm. daily dosages produced a marked drop in blood pressure in eight to nine days, the pressure being 20 to 30 mm. below the control level (fig. 2, curve C). Two of the four rats died after ten daily injections.

DISCUSSION. The systolic blood pressure of the normal rat can be increased from 15 to 50 mm. by the daily oral administration of 1 mgm. of diethylstilbestrol, an effective rise being observed in six to ten days (3). In our experiments the intramuscular injection of the same dosage into normal rats caused an increase in blood pressure but the response was less rapid, being first observed after ten days and definitely established after twenty days. The average pressure increase was 20 mm. and the maximum response was a 38 mm. rise in pressure. The specific manner in which this effect is accomplished is unknown but some possibilities suggest themselves, such as a direct influence on the vascular system, since Reynolds and Foster (4, 5) have observed an effect of estrogen on peripheral circulation although without a constant effect on blood pressure. The adrenal glands may influence the pressure response since these organs were markedly hypertrophied, or the kidney may play an influential rôle in that sterols have been shown by Selye (6) to alter the kidney. Grollman et al. (3), suggest the hypertensive effect is due to renal injury since the elevated blood pressure can be reduced with renal extracts.

In hypophysectomized rats the average systolic blood pressure decreases 30 to 35 mm. during the first ten days post-operatively and maintains approximately the same level for as long as 222 days. However, administration of stilbestrol to hypophysectomized rats causes an initial pressure decrease of 30 to 35 mm. in three days with a further decline of about the same proportion occurring after eight to ten days. That the action of stilbestrol is not merely accentuating the effect of hypophysectomy is shown by a similar reduction in blood pressure in animals in which injections were begun twelve days after the operation. The lowered pressure need not be correlated with death of hypophysectomized rats as we have recorded pressures of 80 to 90 mm. in animals that died during the following twenty-four hours. Thus, under the conditions of these experiments there is no evidence for a direct effect on the vascular system to raise the blood pressure although the toxic effect may overshadow any pressor action. The toxic effect of stilbestrol, in the dosage used, on hypophysectomized rats is further indicated by a maximum ten to fifteen days' survival period whereas in the normal rat a pressor action is evident in this time. The toxic effect of stilbestrol has been observed in adrenalectomized rats (7).

<div align="center">SUMMARY</div>

1. The daily injection of stilbestrol into normal rats produces a gradual rise in systolic blood pressure, reaching hypertensive levels in many cases.

2. The systolic blood pressure of the untreated hypophysectomized rat decreased, on the average, 30 to 35 mm. below normal within ten days after hypo-

physectomy. Rats hypophysectomized for seven months had a blood pressure stabilized at this low level.

3. The injection of hypophysectomized rats with stilbestrol produced a fall in blood pressure below that of operated controls.

REFERENCES

(1) WYMAN, L. C. AND C. TUMSUDEN. This Journal 109: 115, 1934.
(2) WILLIAMS, J. R., JR., T. R. HARRISON AND A. GROLLMAN. J. Clin. Investigation 18: 373, 1939.
(3) GROLLMAN, A., T. R. HARRISON AND J. R. WILLIAMS, JR. J. Pharmacol and Exper. Therap. 69: 149, 1940.
(4) REYNOLDS, S. M. R. AND F. I. FOSTER. This Journal 126: 606, 1939.
(5) REYNOLDS, S. M. R. AND F. I. FOSTER. J. Pharmacol. and Exper. Therap. 68: 173, 1940.
(6) SELYE, H. J. Urol. 42: 637, 1939.
(7) SELYE, H. Endocrinology. 25: 211, 1939.

THE EFFECT OF *l*-ASCORBIC ACID ON EPITHELIAL SHEETS IN TISSUE CULTURE[1]

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The rôle of *l*-ascorbic acid as an activator for the growth of tissues is well recognized. Baker (1) found that the addition of 0.1 to 0.5 mgm. per cent ascorbic acid to a culture medium containing serum, peptone, etc., resulted, after about 2 weeks, in stimulating the proliferation of fowl monocytes. She ascribed the latent period of two weeks to the vitamin reserves in the original tissue. Vogelaar and Ehrlichman (2), by using a largely synthetic medium, found that the stimulating action of ascorbic acid on the growth of mouse sarcoma tissue was appreciable within several days.

Another angle of approach is that of Wolbach and his co-workers (3, 4, 5) who assigned the activity of vitamin C to the elaboration and maintenance of collagenous material which they regarded as cell-binding substances. The deficiency of this interstitial substance in scorbutic animals and recovery on feeding with vitamin C offered a strong argument for this view. Further substantiation has been presented by Jeney and Törö (6) who reported the beneficial effect of ascorbic acid on the formation of fibers in tissue cultures of fibroblasts. Wolbach and Bessey (7), in a recent review, refer to several papers on the beneficial effect of ascorbic acid in the healing of wounds in experimental human ascorbic acid deficiency.

No distinction seems to have been made between collagenous material as a supporting tissue and the actual intercellular cement which binds epithelial cells together. The maintenance of the latter has been shown (8, 9) to be directly proportional to the concentration of calcium ions in the medium. It was of interest to determine whether this effect is stimulated by the presence of *l*-ascorbic acid or whether the latter acts more directly on intracellular reactions.

The feature of the experiments described in this paper is the use of tissue cultures with well-formed epithelial sheets washed free of the diffusible components of the normal plasma medium and then mounted in various experimental media, some of which contained and others lacked *l*-ascorbic acid.

The tissues used were kidney and intestine from 10 day chick embryos, also kidney and parotid gland from 20 day embryos of the guinea pig which is known to depend on external sources for its supply of vitamin C.

The culture medium consisted of 1 part fowl plasma, 3 parts serum (normal guinea-pig serum for guinea-pig tissues and chick serum for chick tissues) and 1 part guinea-pig embryo or chick embryo extract. This clots within 2 or 3 minutes after mixing and forms a soft coagulum around the tissue fragment.

The selected tissues, cut into small fragments (ca 1–2 mm.), were placed in a

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drop of the freshly prepared medium on a coverslip attached to a larger coverslip by a drop of moisture. The larger coverslip, carrying the smaller slip, was then inverted and sealed over a depression slide and the preparation incubated at the appropriate body temperature. This method is known as the double coverslip method (10, 11) and permits transferring the smaller coverslip aseptically and washing the culture undisturbed in its clotted medium.

The experimentation was started after about 48 hours of incubation when the epithelial sheets were extensive. The first step, which involved all the cultures, was to wash them in buffered Tyrode solution[2] for 30 to 45 minutes during which the bathing solution was changed every 4 to 5 minutes. This removed the diffusible ingredients normally present in the clotted medium, but did not affect the fibrin clot which supported the explants and the cellular outgrowths surrounding them. The washing did not disturb the cellular tissues but caused a cessation of growth.

In order to test the possible rôle of l-ascorbic acid on intercellular cement formation, the washing solution, in some cases, was Tyrode solution lacking cal-

TABLE 1

	IMMERSION FLUID	OBSERVED EFFECTS
I	Tyrode solution alone	18 hours: Initial stages of deterioration; granulation of cells and lack of growth
II	Tyrode + cortical extract (1:10,000)	
III	Tyrode + cortical extract (1:1000)	
		48 hours: No improvement
IV	Tyrode + ascorbic acid alone (10 mgm. in 100 cc.)	18 hours: Good condition
V	Tyrode + cortical extract (1:10,000 or 1:1000) and ascorbic acid (10 mgm. in 100 cc.)	48 hours: Good condition

cium salts. This removed Ca ions from the culture until the cells of the sheets became more or less separated by a dissolution of the intercellular cement (9).

The second step was the addition to the culture of the various experimental media. Two general procedures were used to test the effect of l-ascorbic acid on the washed cultures. One was to compare the effect of plasma or serum from a normal guinea pig with that of plasma or serum from a scorbutic guinea pig. The other was to compare the effect of l-ascorbic acid[3] in Tyrode with that of Tyrode alone. In all experiments of this series, the solutions in the l-ascorbic acid were used immediately after preparation. The scorbutic guinea pigs were furnished through the courtesy of Dr. R. L. Zwemer, the College of Physicians and Surgeons, Columbia University[4].

[2] The formula used for the Tyrode solution was: NaCl 8.00 grams, KCl 0.20 gram, CaCl₂ (anhydrous) 0.20 gram, MgCl₂ 0.10 gram, glucose 1.0 gram, NaH₂PO₄ 0.05 gram in distilled water to 1000.00 cc. The solution was adjusted to pH 7.6 by the addition of NaHCO₃.

[3] The l-ascorbic acid was a water soluble crystalline product listed as Ascorbic acid vitamin C, U.S.P. Merck.

[4] The guinea pigs were fed on a diet of oats and showed first signs of scurvy, one on the fifth and the other on the seventh day. Blood for the experiments was obtained on the 27th day when the guinea pigs were significantly ill.

Guinea-pig tissues were used in the experiments with guinea pig plasma and serum, chick embryo tissues in the experiments with the Tyrode solutions.

1. *Experiments with scorbutic plasma and serum.* Forty-eight hour cultures of guinea-pig epithelium were bathed in frequent changes of Ca-free Tyrode solution until the cells of the epithelial sheets were loosened from one another and had become rounded. This occurred in from 20 to 30 minutes. A drop of plasma from a scorbutic guinea pig was then added to the washed cultures. The control cultures were treated in the same way, using plasma from a normal animal.

After 3 hours the sheets had reformed equally well in the experimental and the control cultures. The rounded cells spread out and, when contiguous, cohered into homogeneous sheets with the usual absence of visible cell outlines. Moreover, the sheets remained firmly attached to the culture substrate.

The difference between the effect of the two types of plasma became apparent after 15 hours of incubation. In the cultures with scorbutic plasma, the sheets, although coherent, showed no increase in area and the cells gave signs of deterioration, becoming granular especially along the margins of the sheets where the cells tended to round up and become clumped. On the other hand, the sheets of the cultures with normal plasma grew considerably and appeared normal and healthy.

Similar results were obtained by immersing the cultures in normal and in scorbutic guinea pig sera where they were left for 3 hours at body temperature and then remounted over depression slides each in a hanging drop of the serum in which they had been immersed.

2. *Experiments with l-ascorbic acid in physiological salt solution.* Forty-eight hour cultures of chick epithelium, previously washed in Tyrode, were immersed, some in Tyrode solution alone for controls, and others in Tyrode solution containing 10 mgm. in 100 cc. l-ascorbic acid freshly prepared.[5] One series of the cultures had been washed in the normal calcium-containing Tyrode and another series, in calcium-free Tyrode until the cells had begun to separate.

The sheets with partially separated cells recovered rapidly irrespective of the presence or absence of the l-ascorbic acid in the final calcium-containing medium. However, about 12 hours later the sheets of the cultures in the salt solution lacking l-ascorbic acid were unhealthy and showed signs of degeneration within 18 to 24 hours while those in solutions containing it were healthy and showed active growth for the 96 hours' duration of the experiment. The cultures which had not been previously exposed to the Ca-free solutions gave similar results.

3. *Experiments with adrenal cortical extract.*[6] Experiments were also made to test the action of the whole extract of the adrenal cortex in the presence and absence of l-ascorbic acid on the epithelial sheets of the chick embryo kidney and

[5] No attempts were made to test the effect of varying the concentration of the l-ascorbic acid, although in several experiments a concentration of 5 mgm. in 100 cc. was used with no pronounced differences being noted. The higher concentration was routinely used because of the ease with which the acid is destroyed on oxidation.

[6] The extract was furnished through the courtesy of Dr. E. C. Kendall. One cubic centimeter of this extract represents extractable components of 75 grams of adrenal cortex.

intestine.   The cultures were divided into five lots and immersed in media as indicated in the table.   They were then removed from the media after four hours and sealed over depression slides in hanging drops of their respective experimental fluids.   The results are shown in the accompanying table, viz., that *l*-ascorbic acid stimulates epithelial growth in Tyrode solution irrespective of the presence or absence of the cortical extract.

DISCUSSION.   The experiments described in this paper fully confirm the results obtained by Baker and by Vogelaar and Ehrlichman by demonstrating the importance of *l*-ascorbic acid for stimulating the growth of epithelial tissues. Our more rapid detection of the effect is presumably due to the method of removing effective traces of the *l*-ascorbic acid before starting the experimental procedure.

The failure of *l*-ascorbic acid to affect the development and function of the intercellular cement of parotid gland, intestinal and renal epithelium does not necessarily counter the findings of Wolbach and his co-workers.   Wolbach had shown that vitamin C is essential for the formation and maintenance of interstitial matrices, more particularly of dentin, bone and of collagen in connective tissues.   Menkin, Wolbach and Menkin identified the action described by Wolbach with that of crystalline *l*-ascorbic acid.   The non-essentialness of *l*-ascorbic acid for the production and effectiveness of the material which causes cells to adhere together indicates that the intercellular cement proper is of a different order from materials of collagenous nature.   Cells which depend upon a cement for making them stick together can generate such a cement in the absence of *l*-ascorbic acid.   That which is required to render the cement effective is the presence of ionic calcium which presumably fixes the cement by converting it into a non-dispersible complex.   However, cellular reactions of multiplication, growth and movement are definitely accelerated by the presence of *l*-ascorbic acid.

The experiments with the adrenal cortical extracts were made because of the possibility that these extracts may exert an additive effect to that of the ascorbic acid.   Experiments in this laboratory (unpublished) indicate that the extract of adrenal cortex stimulates the secretory activity of kidney tubules in tissue culture.

SUMMARY

1. Tissue cultures were made of kidney and parotid gland from guinea pig embryos and of kidney and intestine from chick embryos.   The action of *l*-ascorbic acid was tested on the growing epithelial sheets.   Cultures treated with plasma and serum from scorbutic guinea pigs ceased to grow but the epithelial sheets did not lose their coherence.   Ultimately the cells deteriorated.

2. The presence of *l*-ascorbic acid was found to be non-essential for re-establishing the cohesion of epithelial sheets previously separated by the lack of calcium in the medium.   The intercellular cement upon which this cohesion depends must, therefore, be of a different order from the interstitial matrices which,

Wolbach and others have found, depend upon the presence of ascorbic acid for their elaboration.

3. Tissue cultures of epithelium, washed free of the water-diffusible ingredients of the plasma medium and transferred to a buffered salt and glucose solution, remained healthy and active only when *l*-ascorbic acid was present in the solution.

4. The addition of cortical extract either in the presence or absence of *l*-ascorbic acid exerted no observable effect on the viability of tissue cultures.

REFERENCES

(1) BAKER, L.   C. R. Soc. Biol. **121:** 427, 1936.

(2) VOGELAAR, J. P. M. AND E. EHRLICHMAN.   Am. J. Cancer **31:** 283, 1937.

(3) WOLBACH, S. B.   Am. J. Path. Suppl. **9:** 689, 1933.

(4) WOLBACH, S. B.   J. A. M. A. **108:** 7, 1937.

(5) MENKIN, V., S. B. WOLBACH AND M. F. MENKIN.   Am. J. Path. **10:** 569, 1934.

(6) JENEY, A. VON AND E. TÖRÖ.   Virchow's Arch. **298:** 87, 1936.

(7) WOLBACH, S. B. AND O. A. BESSEY.   Physiol. Rev. **22:** 233, 1942.

(8) CHAMBERS, R. AND B. W. ZWEIFACH.   J. Cell. Comp. Physiol. **16:** 255, 1940.

(9) CHAMBERS, R.   Cold Spring Harbor Symposia **8:** 144, 1940.

(10) MAXIMOW, A.   Carnegie Inst. of Washington Publ. Contrib. to Embryol. **16:** 47, 1925.

(11) CAMERON, G.   Essentials of tissue culture technique.   Farrar and Rinehart, New York, 1935.

EFFECT OF HEPARIN ON THE VASOCONSTRICTOR ACTION OF SHED BLOOD TESTED BY PERFUSION OF THE RABBIT'S EAR

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Convincing indirect evidence in favor of the humoral hypothesis of hypertension has made it important to measure quantitatively the presence of specific vasoconstrictor substances in the circulating blood of hypertensive animals and man. For this purpose Page (1, 2) perfused the surviving rabbit's ear with a mixture consisting of equal volumes of Ringer-Locke solution and defibrinated blood from a nephrectomized dog. He found that injecting serum (2) or heparinized plasma (1, 2) from a normal dog or human being into the perfused ear had little or no effect on the blood vessels of the ear, while serum or heparinized plasma from a hypertensive dog or human being was uniformly constrictor. Our attempts to use this technique proved difficult chiefly because of immediate, and irregularly increasing, vasoconstriction which appeared as soon as the diluted, defibrinated blood entered the ear. Moreover, when the constrictor activity of blood plasma or serum from hypertensive and normal animals and man was assayed, the results were highly inconsistent.

Many investigators have reported that serum and defibrinated blood constrict intensely the vessels of isolated perfused tissues (reviewed by Janeway et al. (3) and Amberson (4)). It has been suggested (3) that a constrictor substance is liberated by disintegration of platelets in the course of coagulation. It seemed possible that defibrinated blood as a perfusing fluid, and serum or plasma as samples for assay, might have produced inconsistent results with the rabbit's ear because of varying amounts of powerful vasoconstrictor substances which they contained by virtue of coagulation.

Therefore the present study was arranged *a*, to determine whether defibrinated blood per se produced vasoconstriction in the rabbit's ear; *b*, to observe whether preventing coagulation by large doses of heparin would avoid this constrictor action and thereby produce a more physiological preparation, and *c*, to compare under these conditions the vasoconstrictor properties of defibrinated blood, serum and heparinized plasma from rabbit, dog and man as a necessary step toward using the perfusion method to measure the constrictor activity of blood removed from normal and hypertensive animals and man.

METHODS. a. *Initial technique.* In the first observations, the original technique described by Page (1, 2, 5) was followed. The rabbit's ear was severed rapidly and cleanly with a razor blade and the central artery was immediately cannulated by means of a gauge 21 blunted needle, tied in place by a fine ligature. Within two or three minutes after being severed, the ear was in place in the incubator at 39°C. and the perfusion was started.

The fluids used for perfusion were a, Ringer-Locke solution buffered by phosphate to pH between 7.3 and 7.4, and b, a mixture consisting of one part of defibrinated blood and either one or two parts of Ringer-Locke solution. Defibrinated blood was prepared by stirring whole blood slowly for 30 minutes with several small glass rods rounded at the ends to avoid injuring the corpuscles. After dilution with Ringer-Locke solution the defibrinated blood mixture was filtered through cotton gauze. Both fluids were prepared immediately before use, warmed to 39°C. and placed in suitable flasks in the incubator before the ear was severed.

Intermittent pressure was applied to the surface of both fluids in separate reservoirs by compressed air from a valve rotating about 60 times per minute in conjunction with a reservoir and leak. This pulsatile perfusion pressure could be adjusted between 20/10 and 140/110 mm. Hg. Connections were arranged so that transfer from Ringer-Locke solution to defibrinated blood could be made without any interruption of flow.

Immediately after the ear was mounted the buffered Ringer-Locke solution was first perfused at a pressure of 40/30 mm. Hg or less until rapid and constant flow indicated that the initial vasoconstriction due to trauma had disappeared and that the ear was properly fixed in its final position. Then perfusion by defibrinated blood was started. The perfusion fluid entered the central artery, passed through the vessels of the ear and flowed from the cut veins at the base of the ear, to be collected in a funnel from which it passed dropwise over the terminals of an electrical drop recorder. A kymograph record showed time in minutes, mean perfusion pressure and the number of drops. Fifteen drops had a volume of 1.0 cc. The air within the incubator was kept moist by means of filter paper or cotton saturated with water.

b. *Modified technique.* Owing to increasing and variable vasoconstriction observed with this initial method, heparinized blood was then used in place of defibrinated blood. Between 35 and 45 cc. of blood was withdrawn from the heart of a rabbit into a 50 cc. syringe containing 1.2 cc. Connaught heparin (1000 units per cc.). Blood and heparin were mixed throughly by inverting the syringe several times. The heparinized blood was diluted immediately with two volumes of Ringer-Locke solution and filtered through cotton gauze. This perfusion mixture was also often constrictor, as will be described under "observations", and the following modifications were finally adopted routinely. It appears that each factor is essential because omission of any one was followed sooner or later by grossly irregular results.

1. *Thorough heparinization.* An excess of heparin was used at each step in preparing the perfusing fluid to avoid coagulation as completely as possible. One and five-tenths cubic centimeter of Connaught heparin solution was injected into the donor rabbit intravenously, fifteen minutes before blood was drawn. For rabbits weighing more than 3 kilos, 1.8 cc. were used. Fifteen minutes later, between 40 and 45 cc. of blood were withdrawn by cardiac puncture into a syringe containing 1.2 cc. of heparin solution and mixed as usual. This blood was then diluted immediately with two volumes of warm Ringer-

Locke solution to which heparin solution had previously been added in the amount of 0.3 cc. in 100 cc. The mixture was filtered through cotton gauze previously saturated with heparinized Ringer-Locke solution and then placed in the incubator ready for perfusion. As an additional safeguard during perfusion, 0.3 cc. heparin solution was added at the end of 30 minutes to such blood as still remained in the reservoir awaiting perfusion. Blood prepared in this way will be called "pre-heparinized blood."

2. *Oxygen and carbon dioxide.* A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was led to the perfusion flask and was bubbled very slowly through the perfusing mixture to keep the erythrocytes from settling in the reservoir and to approach normal gaseous equilibrium.

3. *Precautions against cooling.* For dependable results it proved necessary to make all injections without opening the incubator. This was accomplished by piercing the rubber tube, which led the perfusion fluid to the ear, with a minute T-cannula consisting of a gauge 20 needle with gauge 20 side-arms. Rubber tubing with 0.5 mm. bore was passed from these two side-arms to the outside of the incubator through the wall. To one of these tubes was connected the syringe for making injections while the other was closed with a clamp. Prior to each injection the clamp was opened and the solution to be tested was washed from the syringe through one rubber tube past the base of the T-cannula, and out through the other rubber tube. The solution having thus been brought to the base of the T-cannula, the clamp was closed and exact volumes could be injected into the perfusion fluid from outside the incubator without chilling the ear.

4. *Careful cleansing.* According to Janeway (3) the powerful constrictor substance produced in the course of coagulation of blood is destroyed by alkali and dissolved by alcohol. Therefore, after each experiment, all glassware and tubing were completely dismantled and not only thoroughly washed with soap and water but soaked at least ten minutes, and preferably an hour, in 95 per cent alcohol to remove any vasoconstrictor material produced by traces of blood left in the tubing or its joints. All syringes and needles were cleansed similarly. At intervals, also, all parts were boiled in saturated sodium bicarbonate solution. After each cleansing, fresh Ringer-Locke solution was washed through the entire apparatus in large quantities.

OBSERVATIONS. 1. *Comparative effects of defibrinated, heparinized, and "pre-heparinized" blood on vascular tone in the rabbit's ear.* The differences in the vasoconstrictor activity of three types of shed blood are illustrated in figure 1. In these observations the ear was perfused as usual with Ringer-Locke solution for 5 or more minutes until the rate of dropping was rapid and constant (not shown in fig. 1). At zero time the perfusion with Ringer-Locke solution was stopped and the blood mixture began entering the ear. The rate of dropping was observed for 60 minutes or until perfusion was practically stopped by tight vasoconstriction. Perfusing pressure remained constant throughout so that decrease in drop rate indicated constriction.

When defibrinated blood entered the ear at a pressure of 40/30 mm. Hg, the

whole ear became pink but almost immediately turned pearly white as vaso-constriction reduced the rate of flow almost to zero. The cessation of flow was sudden enough to suggest mechanical obstruction by thrombi. That this was not the case was demonstrated by perfusing alternately with defibrinated blood and with Ringer-Locke solution. For three ears, Ringer-Locke solution at a pressure of 40/30 mm. Hg flowed initially at rates between 38 and 45 drops per minute. Changing to defibrinated rabbit's blood reduced the perfusion rate within two minutes to between 1 and 3 drops per minute. Returning to Ringer-Locke solution re-established perfusion at rates between 35 and 45 drops per minute but complete recovery required 10 minutes. A second and third shift to defibrinated rabbit's blood again reduced the drop rate to between 0 and 2
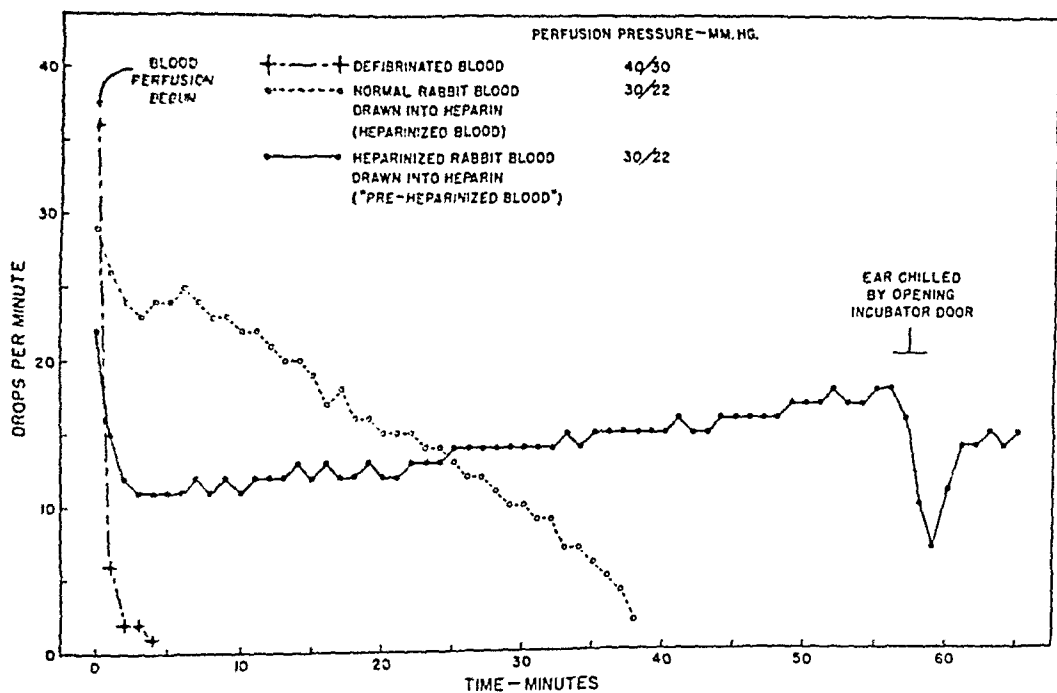
Fig. 1. Effect of perfusion by defibrinated blood, heparinized blood and "pre-heparinized blood" on the tone of the vessels of the rabbit's ear. To right is shown effect of cooling the ear.

drops per minute after which return to Ringer-Locke solution after about 10 minutes restored the original perfusion rate. This repeated reversibility indicates that slowing of flow was due to constriction rather than to mechanical obstruction by thrombi.

As shown in figure 1, when perfusion was performed with heparinized blood, the rate of flow decreased somewhat at the very beginning as would be expected because of the greater viscosity of the blood mixture as compared to Ringer-Locke solution. Thereafter for ten to twenty minutes a fairly adequate perfusion rate was observed and the ear looked generally pink and healthy. Shortly, however, the perfusion rate began to fall more or less regularly. The central artery and veins gradually became smaller until finally the ear was

pearly white, perfusion rate was low and the end result resembled that produced much more rapidly at these low pressures by defibrinated blood.

In contrast to these mixtures "pre-heparinized blood" (solid line, fig. 1) produced the usual preliminary drop in perfusion rate due to change in viscosity of the perfusing fluid, but the ear remained pink, and the central artery and marginal veins stayed moderately dilated. Moreover, the perfusion rate remained relatively high and constant at 12 to 20 drops per minute with perfusing pressures not above 40/30 mm. Hg.

Rabbit's ears perfused at low pressure with "pre-heparinized blood" proved to be extremely sensitive to minute changes in temperature. If the incubator door was opened briefly, a fall of 3 or 4°C. in air temperature often produced a 50 to 80 per cent drop in perfusion rate lasting from 4 to 6 minutes, as shown to the right in figure 1. When control injections of Ringer-Locke solution were made by opening the incubator door there usually followed a constriction which was due not to the fluid injected, but to coincident cooling. Avoiding this artefact is highly important in assays of constrictor action.

In early attempts to apply the method of Page (1, 5) to the assay of constrictor substances in human or dog blood many types of perfusion mixtures, containing serum, plasma, or washed cells, were tested. Results similar to those for defibrinated rabbit's blood were obtained with rabbit serum, defibrinated dog's blood, and with some samples of heparinized blood from either dogs or rabbits. Results more or less similar to those for heparinized rabbit's blood were observed with most samples of heparinized dog's blood, washed rabbit or dog erythrocytes resuspended in Ringer-Locke solution or with quickly separated plasma from heparinized dog's or rabbit's blood. To obtain a drop rate of 15 per min. pressures of 50/30 up to 125/100 were often necessary, indicating greatly increased tone due to the perfusing fluid itself. Defibrinated blood, heparinized blood and heparinized plasma obtained from rabbits or dogs nephrectomized 24 or 48 hours previously yielded results similar to those obtained with blood from normal animals.

These observations all indicated that, as in other isolated tissues (4), the perfusion of the isolated rabbit's ear with defibrinated or simple heparinized blood is accompanied by vasoconstriction. To avoid this effect it is essential to guard against coagulation at every step. To determine to what extent this error might interfere with the assay of pressor substances of renal or other origin in the circulating blood, various types of blood samples from rabbit, dog and man were tested for their constrictor activity by injecting small volumes such as are used for assay.

2. *Vasoconstrictor effects of small amounts of shed blood or its fractions.* In these observations the ear was perfused at low pressure with "pre-heparinized" blood. After a suitable control period, usually 5 to 10 minutes, 0.2 cc. of shed blood or its fractions was injected through the T-cannula into the perfusion mixture as it passed through the rubber tubing immediately before entering the central artery of the ear. Rabbit's blood was used in this series of experiments to eliminate effects due to species differences. Perfusion pressure was kept constant throughout each experiment.

Figure 2 illustrates typical results of numerous observations of this kind. The constriction produced by simple cooling of the ear is shown in figure 2A. Control injections of Ringer-Locke solution (fig. 2B) had no effect on perfusion rate providing the injections were made from outside the incubator. Full strength Connaught heparin solution, as supplied in ampules commercially, did not affect vascular tone when it was injected alone in volumes of 0.2 (fig. 2C) or even 0.6 cc. Defibrinated rabbit's blood produced immediate and complete constric-
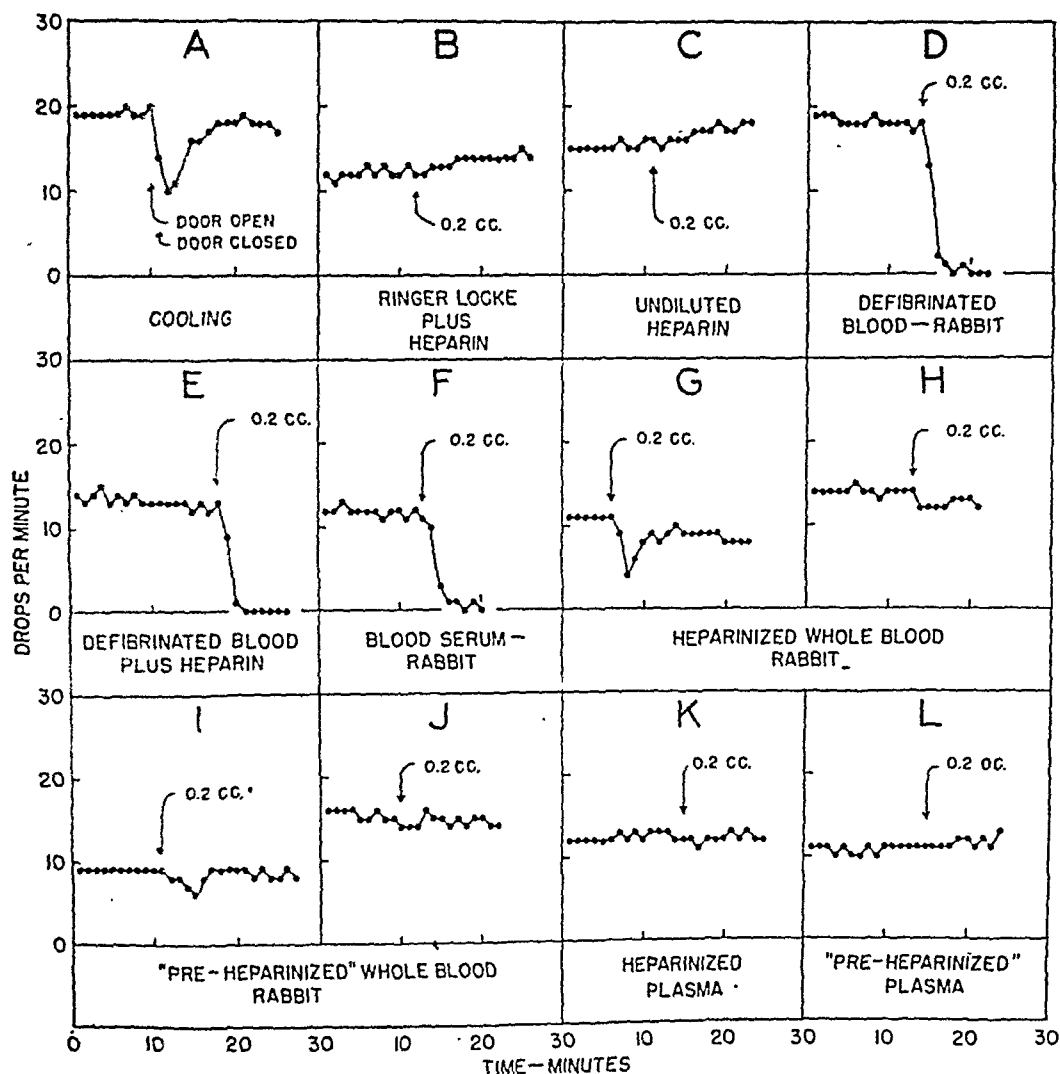
Fig. 2. Effect of cooling, heparin, and of certain types of shed blood on the tone of the vessels of the rabbit's ear.

tion (fig. 2D) which lasted at least 7 or more minutes in 6 observations. Heparin, 0.5 cc., was then added to 10 cc. of defibrinated blood and the mixture was incubated for 30 to 45 minutes without affecting the intense constriction produced by injecting 0.2 cc. (fig. 2E). This indicates first, that once vasoconstrictor substance had been released by coagulation, heparin did not neutralize or destroy it, and second, that heparin per se had no detectable dilator effect under these conditions. Blood serum (fig. 2F) also produced complete constriction and cessation of flow. Both defibrinated blood and serum changed the

color of the ear from the normal pink to waxy white as the previously dilated arteries and veins narrowed and then became imperceptible.

Heparinized whole blood, i.e., blood drawn from the heart of a normal rabbit into a syringe containing heparin, was slightly constrictor (fig. 2, G and H) in almost every instance. "Pre-heparinization" of the donor rabbit diminished still further the development of constrictor activity in whole blood in that doses of 0.2 cc. had very slight or no effect on perfusion rate (fig. 2, I and J).

Constrictor activity was least in plasma separated immediately after the blood was withdrawn from the heart of a normal or a heparinized rabbit (fig. 2, K and L). In preparing this "heparinized plasma" (fig. 2K) and "pre-heparinized plasma" (fig. 2L), blood from the heart of a normal or heparinized rabbit respectively was drawn into a syringe containing the usual 1.2 cc. of heparin solution. After mixing, the blood was rotated in plastic (Lusteroid) tubes in an angle centrifuge at high speed for 5 minutes. The upper layer of plasma was decanted into another tube and centrifuged again for 10 minutes. This was decanted and placed in the incubator for injection. As shown in figure 2K and L, constrictor substances were usually absent. If Janeway's suggestion that disruption of platelets is responsible for the vasoconstrictor activity is correct, it would be expected that this quickly separated, and doubly centrifuged, plasma would have least effect on the tone of the perfused vessels because most of the platelets are separated from the plasma before there is opportunity for gradual disintegration.

3. *Quantitative comparison of the constrictor activity of samples of rabbit's blood collected in various ways.* Full curves for rabbit's serum, heparinized blood and plasma from heparinized blood are shown in figure 3. After injection, flow quickly reached a minimum followed by gradual recovery. Perfusion pressure remaining constant, the total constrictor effect is indicated by the degree to which rate of flow is diminished and by the time required for flow to return to the control level. The scale for charting was always one inch for 10 minutes and one inch for ten drops. Rates of flow were kept as closely as possible between 10 and 15 drops per minute by adjusting pressure between 26/16 and 40/30 mm. Hg at the very beginning of each experiment and the pressure was kept constant thereafter for each ear.

Under these conditions the whole effect of a given injection can be expressed most simply by measuring the area enclosed between the observed rate of flow (solid lines, fig. 3) and the expected rate of flow without injection (dotted lines, fig. 3). Errors in this schematic method of expressing constrictor activity were relatively unimportant compared to the magnitude of the observed differences which were quite obvious to simple inspection of the original charts. Thus in figure 3 the greatest constrictor effects produced by plasma from heparinized blood and by heparinized whole blood were clearly much less than the smallest constrictor effect produced by serum in like dosage. The areas given in table 1 are used to avoid publishing many figures of the curves themselves.

Table 1 shows the average effects of control injections (Ringer-Locke solution) and of variously prepared samples of blood. The average intensity and duration

of constriction is indicated to the left while the last column to the right gives the average area of the curves showing change in flow of perfusate. The negative sign shows that the sample produced constriction and reduced flow; the figures in parentheses indicate the range of variation.

Control injections of Ringer-Locke solution had no effect. Defibrinated blood and serum were uniformly highly constrictor. It seemed possible that if blood were removed from the donor rabbit without using any anticoagulant and
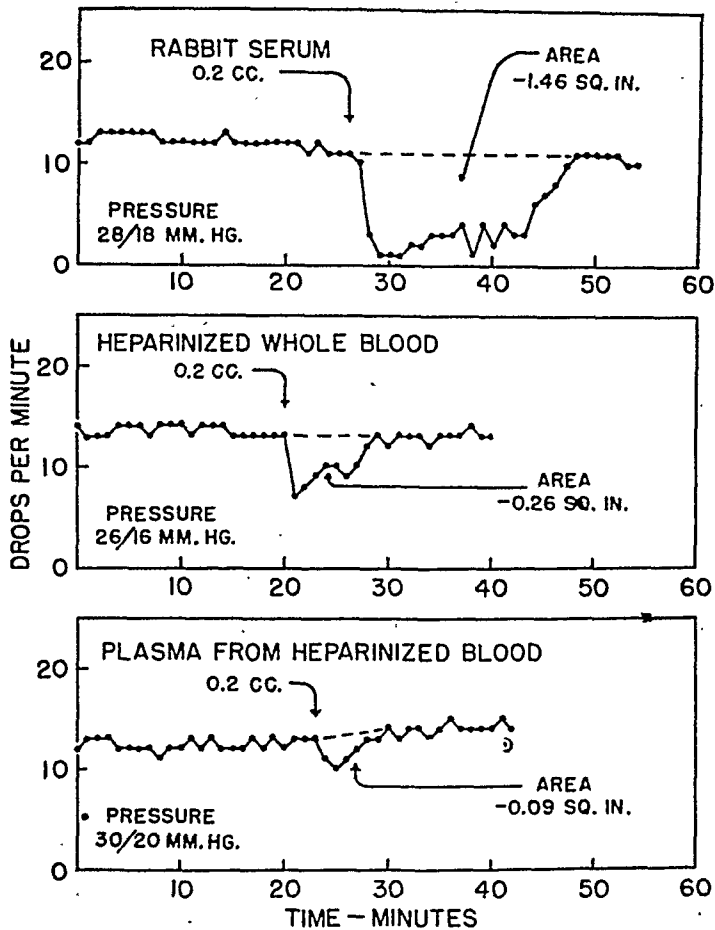
Fig. 3. Complete curves of flow showing effect of 0.2 cc. of rabbit serum, heparinized whole blood and plasma from heparinized blood on flow through the vessels of the rabbit's ear. Also shown is method of computing constrictor effect by area. For serum, 1.46 sq. in. was the *smallest* area observed while for heparinized blood and plasma, respectively, 0.26 and 0.09 sq. in. were the *greatest* areas observed.

injected within one minute or less, constrictor action might be avoided because in this brief period the blood remained fluid and did not have time to coagulate. As shown in table 1 the intensity and duration of constriction were greatly reduced but the areas of the curves were too variable to permit accepting this method as a dependable one for assay purposes. Samples of heparinized and pre-heparinized whole blood, even after standing in the incubator for one hour, were approximately one-tenth as constrictor as serum and roughly one-half as

constrictor as whole blood injected immediately. The variations were still marked.

On the contrary, samples of plasma separated rapidly by centrifugation from heparinized or "pre-heparinized" blood were usually inert and only occasionally very slightly constrictor. The greatest areas observed with these samples of plasma were 0.09 and 0.02 sq. in. respectively and represent changes of doubtful significance because control curves may show variations of this grade. The

TABLE 1

Effects of 0.2 cc. injections of rabbit's whole blood, serum and plasma on vascular tone in the rabbit's ear perfused with "pre-heparinized" rabbit's blood

1	2	3	4	5	6
	NUMBER OF INJECTIONS	AVERAGE RATE OF FLOW, CONTROL PERIOD	MAXIMAL REDUCTION OF FLOW AFTER INJECTION	AVERAGE TIME FOR FLOW TO RETURN TO NORMAL	AREA OF DECREASED FLOW CURVE
		drops per min.	*per cent*	*mins.*	*sq. in.*
Control injections Ringer-Locke soln..	5	17	0	0	0.00
Defibrinated blood......................	6	17	100	9*	−1.61* (1.05–2.66)
Serum.................................	5	11	98	28	−1.95 (1.46–3.85)
Whole blood injected within one minute...........................	17	13	52	6	−0.25 (0.00–0.81)
Heparinized whole blood..............	8	11	43	5	−0.14 (0.00–0.26)
"Pre-heparinized" whole blood........	7	12	42	5	−0.15 (0.00–0.39)
Plasma from heparinized blood........	4	14	9	2	−0.04 (0.00–0.09)
Plasma from "preheparinized" blood..	6	14	1	0.3	−0.01 (0.00–0.02)

* Minimum figure since recovery had not even started when the observations were terminated at 7 to 11 minutes. For serum and all other blood samples areas are based on the entire curve, continued until the control rate of flow returned.

average areas for heparinized and "pre-heparinized" plasmas were even smaller, 0.04 and 0.02 sq. in. respectively.

It seems clear that "pre-heparinized", quickly separated plasma has less constrictor activity than any other type of blood sample from the rabbit. Pre-heparinization is expensive and, for man, impracticable. Quickly separated, heparinized plasma is almost as inert and therefore observations on these samples were extended to the dog and man.

4. *Comparison of constrictor activity of defibrinated blood, serum and quickly separated heparinized plasma from rabbit, dog and man.* To ascertain the applicability of the rabbit's ear technique for the study of bloods of other species

the comparison in table 2 was made. Blood was removed from superficial veins of dogs or man and then treated as described above. It is obvious that defibrinated blood and serum of all three species are highly constrictor and that heparinized plasma is almost inert. In the experiments with dog plasma, 4 injections of heparinized plasma from a single animal were slightly constrictor on one day, but the next day, technique being presumably identical, a second sample of heparinized plasma was entirely inert in 4 injections. The reason for this difference is not clear, though probably due to early and slight coagulation. Even when this observation is included the average constrictor action of dog's heparinized plasma is only one-twentieth that of serum.

TABLE 2

Comparison of effects of defibrinated blood, serum and heparinized plasma from rabbit, dog and man

	RABBIT	DOG	MAN
Defibrinated blood			
No. obs.	6	9	9
Constr. eff. in sq. in.	−1.61*	−3.48	−1.60
Serum			
No. obs.	5	7	11
Constr. eff. in sq. in.	−1.95	−5.05	−3.83
Heparinized plasma			
No. obs.	4	19	16
Constr. eff. in sq. in.	−0.04	−0.24†	−0.07
		(−0.01)	

* See footnote, table 1.

† Including one divergent experiment. Figures in parentheses show result when this one experiment is excluded.

DISCUSSION. To perfuse isolated tissues or organs under optimal physiological conditions requires the use of oxygenated blood under pulsating pressure. Yet practically all investigators have found that shed blood after a short time induces an intense vasoconstriction which has been lessened by inserting the lung (6, 7, 8, 9, 10), liver or spleen (11) into the perfusion circuit, or by adding ergotoxine to the perfusate (7, 11). Such methods are not suitable for assay of pressor substances because of complicated technique and partial effectiveness in the first instance and because of transient pressor action of ergotoxine itself in the second instance. The hypothesis that defibrinated blood flows with great difficulty owing to mechanical block by coagulated particles (12) has been disposed of by Bayliss and Ogden (7) who found that filtration through cambric did not ensure rapid flow and by Janeway (3) who described a diffusible, heat stable constrictor substance arising from platelets. Moreover, as observed in the present studies, perfusion of the ear alternately by defibrinated blood and Ringer-Locke solution showed that flow, though stopped completely, would return to normal repeatedly when the defibrinated blood was washed out of the ear. This could not occur if mechanical block were an important factor.

Constriction of isolated arterial strips or of vessels in situ has been produced by defibrinated blood, by serum (3, 6, 7) and by blood made incoagulable with oxalate, citrate, hirudin (3) or heparin (7). Janeway (3) concluded that uncoagulated blood, while circulating in the intact animal, is not constrictor but that immediately after it is removed from the circulation, and before it becomes thoroughly mixed with the anticoagulant solution, a powerful constrictor substance appears. In agreement with this concept, the perfusion of the rabbit's ear with defibrinated blood produced striking vasoconstriction and unphysiologically slow rates of flow even at high pressures. Page's results, though pressures are not given for individual perfusions, also seem to agree with this conclusion. At perfusion pressures of 140/110 mm. Hg with recirculation the tabulated rates of flow ranged from 18 to 42 drops per minute (6). In a later paper (2) with pressures of 60/40 to 80/60 mm. Hg rates ranged from 9 to 29 drops per minute. In a third series (1) pressure was not specified and the rate of flow ranged from 3 to 36 drops per minute.

For the rabbit's ear simple heparinization of whole blood reduced, but did not abolish uniformly, this progressive vasoconstriction in agreement with Bayliss and Ogden (7) who found "vasotonins" in heparinized blood. Finally with "pre-heparinization" perfusion rates ranged from 8 to 42 drops per minute at pressures of 40/30 to 26/18 mm. Hg. Equally important was the greater constancy of flow in any one preparation over the usual period of one to three hours. If additional donor rabbits were sacrificed, it was possible to continue perfusion for even longer periods without edema and without lessened response to 1:1,000,000 epinephrine. However, since 0.2 cc. of "pre-heparinized" whole blood sometimes produced slight but definite vasoconstriction (table 1) this perfusion medium is still not absolutely physiological, though far superior to defibrinated blood.

It is possible that smaller amounts of heparin would have sufficed but a large excess was used because of the nature of heparin's action (13) and because amounts much greater than those used routinely in perfusion had no effects upon the tone of either dilated or constricted vessels. The minute amount of phenol contained in liquid heparin as a preservative did not affect the results because pure heparin powder (Connaught) dissolved in salt solution and used in similar unitage was quite as effective. Another commercial heparin, Liquaemin-Roche, was tested but its anticoagulant activity per unit volume was far less and its action was correspondingly less dependable.

Dicoumarin[1], or 3,3'-methylenebis-4-hydroxycoumarin, given orally to 10 rabbits for 3 days in doses of 100, 50 and 50 mgm. reduced the vasoconstrictor activity of their shed blood almost as effectively as did "pre-heparinization." It seems likely that the effects of heparin on constrictor activity are due to arrest of coagulation at an early stage, rather than to any specific action of heparin per se.

The minimal constrictor activity of heparinized or "pre-heparinized" plasma and the marked constriction produced by serum differ radically from the results

[1] Dicoumarin was supplied by courtesy of Abbott Laboratories.

of Page (2) who found that serum and plasma of normal or of hypertensive dogs were equally inert when injected into a rabbit's ear perfused with Ringer's solution or with defibrinated blood from a normal dog, while serum and plasma of hypertensive dogs or man were both constrictor when injected into an ear perfused with defibrinated blood from a nephrectomized dog. No explanation of this discrepancy can be offered from our observations.[2]

Preliminary studies in which the rabbit's ear was perfused with "pre-heparinized" rabbit's blood indicate the quantitative significance of constrictor substances from coagulation in assays of circulating constrictor substances by perfusion methods. For a given volume of blood the constrictor activity that appears in the course of coagulation is several times greater than that which can be produced by mixing the same volume of blood with an excess of renin which transforms all the available renin activator into the constrictor substance called angiotonin by Page (1). Under these circumstances it appears extremely important to exclude artefacts due to early coagulation whenever the amount of constrictor substance in the circulating blood is measured by injecting samples of shed blood into an isolated perfused tissue.

CONCLUSIONS

In agreement with previous workers who used other tissues, it was found that defibrinated blood as a perfusion fluid induces marked vasoconstriction in the surviving ear of the rabbit. Heparinized blood was moderately constrictor.

Perfusion of the rabbit's ear could be accomplished with less progressive constriction by injecting heparin into the donor rabbit to arrest coagulation at an early stage before the blood was withdrawn. Precautions against cooling of the ear were also essential for consistent results.

Small amounts, 0.2 cc., of defibrinated blood and serum from normal rabbits, dogs and human beings induce marked constriction. Heparinized or "pre-heparinized" whole blood is much less constrictor but still too variable to be useful in assays.

Plasma rapidly separated from heparinized or "pre-heparinized" blood of normal rabbits, dogs and human beings has little or no vasoconstrictor activity.

These studies emphasize the importance of excluding coagulation when the rabbit's ear method is used to detect constrictor substances of renal or other origin in the circulating blood.

For technical assistance we are indebted to Mr. Herman Goslyn and to Mr. J. E. Wood, III.

[2] Page (14) in a letter dated January 7, 1943 enclosed the following comment to be added as a footnote: "Our method was originally designed to avoid the occurrence of vasoconstrictor substances resulting from coagulation of the blood. Hence my statement in a recent article (2) that serum and plasma can be used interchangeably is completely erroneous. This is the only time that the error has occurred so far as I can find. I confess that I am as puzzled as Dr. Landis as to why the mistake occurred and can account for it only on the basis of a careless misstatement."

REFERENCES

(1) PAGE, I. H.   J. Exper. Med. **72**: 301, 1940.

(2) PAGE, I. H.   Am. Heart J. **23**: 336, 1942.

(3) JANEWAY, T. C., H. B. RICHARDSON AND E. A. PARK.   Arch. Int. Med., **21**: 565, 1918.

(4) AMBERSON, W. R.   Biol. Rev. **12**: 48, 1937.

(5) PAGE, I. H.   J. Exper. Med. **70**: 521, 1939.

(6) STARLING, E. H. AND E. B. VERNEY.   Proc. Royal Soc. London (Series B) **97**: 321, 1924.

(7) BAYLISS, L. E. AND E. OGDEN.   J. Physiol. **77**: 34P, 1933.

(8) EICHOLTZ, F. AND E. B. VERNEY.   J. Physiol. **59**: 340, 1924.

(9) HERRICK, J. AND J. MARKOWITZ.   This Journal **88**: 698, 1929.

(10) HEMINGWAY, A.   J. Physiol. **71**: 201, 1931.

(11) BING, R. J.   This Journal **133**: 21, 1941.

(12) DALY, I. DE B. AND W. V. THORPE.   J. Physiol. **79**: 199, 1933.

(13) FERGUSON, J. H.   Proc. Soc. Exper. Biol. and Med. **42**: 33, 1939.

(14) PAGE, I. H.   Personal communication, dated January 7, 1943.

PATTERN OF NORMAL WATER DRINKING IN DOGS

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Intake of water is a discontinuous process; in contrast to output of water which is continuous, and ordinarily almost constant in rate from minute to minute. The discontinuity suggests that the water content of the body periodically becomes sensibly unbalanced, whereas the act of water drinking restores the body and relieves at once the maladjustment of content. What sort of signal initiates water drinking in hourly life? How regular are the periods at which it operates? What sorts of factors modify the periods?

One of nine trained dogs was put in a stall suspended from a Sauter balance. Most observations were made upon two females with exteriorized bladders. In periods of 0.25 hour the insensible loss of weight was ascertained. Urinary loss was found by measuring the urine collected from the exteriorized bladder each 0.25 hour. In most periods, drinking water was available in a can just in front of the dog. Whenever the dog took water, the can and its contents were reweighed. Room temperatures lay between 22° and 26°C. and relative humidities between 30 and 50 per cent.

In some tests the dog did not merely stand in the stall loosely confined, but heat was applied or food was given. Heat was added to the body by two radiators placed laterally at constant position and intensity. The intensity was chosen such that the dog did not fidget. Food consisted of dried whole milk and fox chow, usually mixed with an equal weight of water. The dog swallowed at once all of the food offered. On most days the food allowed each 24 hours was constant, the amount being sufficient for maintenance of body weight.

The Sauter balance was used in such a manner that exact equilibrium between its two sides was not required. One minute before the dog's weight was to be ascertained, the balance was allowed to swing, with approximately correct weights in the pan. The midpoints of the free swings just before and just after zero time were averaged; and since the deflection per gram had been previously ascertained with the same dead loads on the balance, the weights in the pan could be corrected, to give an accuracy of weighing of 0.2 gram. Actually only the nearest 0.5 gram differences were ascertained, since these gave 6 per cent accuracy per 1-hour period at the slowest rate of insensible loss.

In each test the dog had been fed 16 hours previously and had been allowed water *ad libitum.* In addition, the dog was offered water upon coming to the stall; if it drank some, drinking to satisfaction was allowed and the test was started; but if it drank none, the dog was not considered to be in a reproducible state of water balance and no test was run upon it that day.

A. *Control.* When water was continuously available, rates of water loss be-

came stabilized about 1 hour after the dog was first weighed (fig. 1). In this period the animal "calmed down" and slight diuresis resulted from the water spontaneously drunk at zero time.

Water was voluntarily drunk in only one instance during ten control tests that lasted 2 hours, in which time 0.35 per cent of the body weight (B_0) had been lost (A, fig. 2). In the one instance 0.29 per cent of B_0 was drunk at 1.6 hours, just restoring the body weight. In two tests that lasted 4 hours, water was taken once at 3.7 hours to the amount of 0.40 per cent of B_0. During the 4 hours 0.60 per cent of B_0 had been lost.
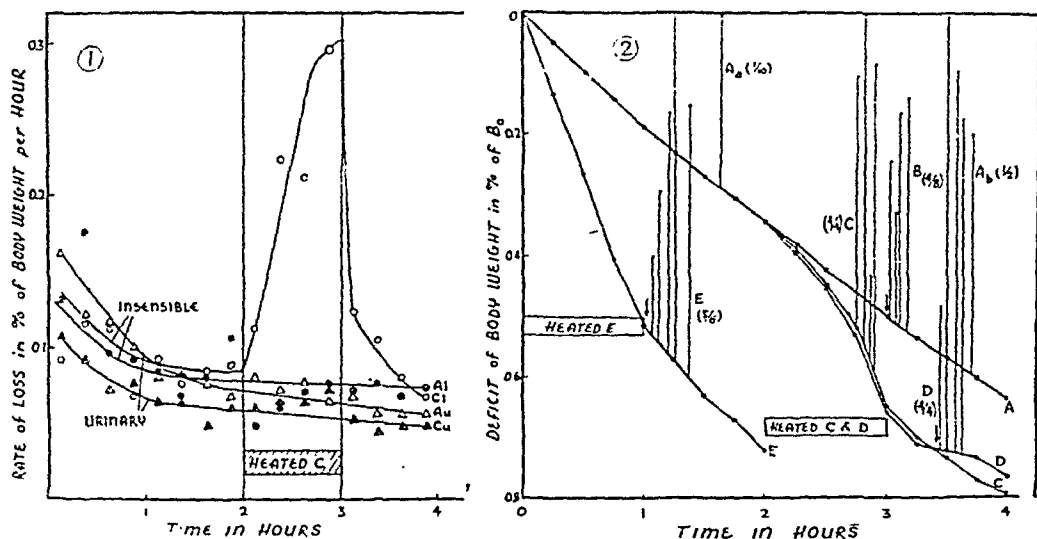
Fig. 1. Rates of water loss from a dog standing in stall. A, mean of two control tests. C, mean of three tests in which radiant heat was applied for 1 hour.

Fig. 2. Deficits of body weight of dogs, in per cent of initial weight, in various tests mentioned in the text. Vertical lines indicate water voluntarily drunk. In tests A and C water was always available; in other tests it was allowed after the arrows. A_a, control for 2 hours; A_b, control for 4 hours. B, water not allowed for 3 hours. C, control for 2 hours, then heated for 1 hour. D, control for 2 hours, then water not allowed for 1½ hours while heated in third hour. E, water not allowed for 1 hour while heated. Fractions, 1/10, 5/6, etc., indicate number of tests in which water was drunk out of the total tests.

The pattern of ordinary drinking is plain. The dog does not sip water at frequent intervals even though the water is immediately available, but waits some hours between drafts. The draft when taken is not sufficient to restore body weight, but may be considered sufficient to restore water content relative to the body's content of other substances.

B. *No water; later allowed.* When water was not allowed during the first 3 hours, weight was lost as before. Then water was placed before the dog, and in four of eight tests water was drunk within a few minutes. The amounts ingested varied only between 0.18 and 0.38 per cent of B_0, 0.50 per cent of B_0 having been lost. Comparison with the previous result possibly indicates that lack of availability of water tends to induce drinking more frequently when later the water is allowed. But in no case is the amount drunk sufficient to restore body weight.

On the average a loss of weight equal to 0.5 per cent of B_0 is required before drinking supervenes.

C. *Control, then heat.* In these four tests, water was available at all times. After 2 hours of control conditions, heat was applied for 1 hour, the rate of weight loss being greatly accelerated. In the last half of this hour, water was drunk in every instance, and in amounts not sufficient to restore the body weight. Again, 0.5 per cent of B_0 had been lost before drinking occurred (C, fig. 2).

Rectal temperatures fell during the control hours, rose about 0.8°C. during the heating, and fell during 1.3 hours thereafter. The drinking was not clearly related to any particular rise of body temperature.

D. *Heat; water later.* The same form of test was modified by denying the dog water during the heating and for 0.5 hour thereafter. Would the fact that heat no longer impinged on the body lessen the urge to take water? The answer was that just as much water was drunk as in C, in the four tests. Therefore, the heat does not exert its influence only immediately, but also latently. Altogether, heating was scarcely more efficacious than non-heating in induction of water ingestion, at the same deficit of body weight.

E. *Initial heat; water later.* Water was denied after the initial drink of the day. Without a previous control period, heat was applied for one hour (E, fig. 2). Then water was allowed, and in five of six tests was drunk within 0.5 hour. In this series 0.5 per cent of B_0 had been lost in 1 hour instead of 3 hours; no more water was taken here than there. It appears that time is a negligible variable; the amount of water missing from the body is the factor in common.

Summary. Dogs placed under control conditions with minimal physical activities, gradually lost weight by evaporation and by urine formation. Periodically these losses were partially made up by drinking. Drinking rarely occurred before 0.5 per cent of B_0 had been lost, and was insufficient to restore body weight. It occurred somewhat more surely when water had been previously denied the dog, and when the loss had been hastened by heating. The heating may have been over and gone before water was offered, yet drinking occurred more freely. The chief factor in inducing drinking appears to be the lack of body water and not the time that intervened during its loss.

F. *Control with food.* It is shown above that dogs which were fed many hours ago drink little water. This fact has long been recognized in that dogs drink only $\frac{1}{10}$ to $\frac{1}{3}$ as much water upon days when food is withheld as upon days of usual feeding.

In order to study the effects of eating upon drinking, food as well as water was given *ad libitum* at all hours. A popular notion is that dogs cannot be permitted unlimited food; the regime, however, proved satisfactory. The dogs ate somewhat more than was required for maintenance of weight, gaining about 0.5 per cent of B_0 per day on the average.

In these tests (32 days) two dogs remained in metabolism cages. All food and water present were automatically registered. Water level in the drinking pan was recorded as previously described (Adolph, 1939). Dry food was kept in a pan suspended from a spring within the dog's cage; a string from the pan to a

lever indicated the length of the spring and consequently the weight of the food remaining.

The food was taken in about five meals per day. No fixed hours were evident for these meals; but more than average occurred in late afternoon when the dogs were accustomed to being fed, and very few occurred late at night. Only rarely was food merely tasted; instead, eating continued for 5 minutes or more and a mode of 0.18 per cent of B_0 was swallowed at each occasion. Water was rarely drunk between bites, but usually was taken only after eating was finished, when modally 0.38 per cent of B_0 was ingested within 10 minutes. The pattern of water drinking therefore shadowed that of eating. Body weight was fully restored at most meal times. Hence, the periodic taking of food with water eliminated the trend of decrease in body weight that prevailed in the absence of food. Instead, ingestions, which voluntarily occurred every 4 to 6 hours, restored body weight and made it oscillate about a gradual upward trend.

G. *Food at stated intervals.* The relation of food intake to drinking was studied further by allowing portions of food at fixed intervals. Whereas the
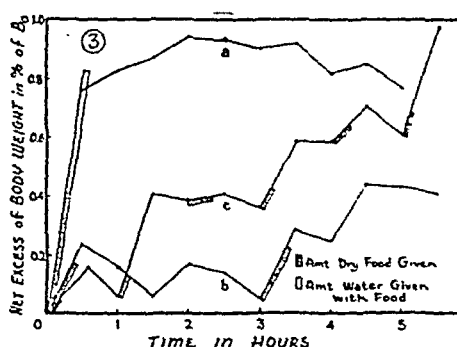
Fig. 3. Excesses of body weight of dogs, in per cent of initial weight, in tests G in which food was periodically given. The weight of food allowed is indicated by thickened lines; all other gains of weight were due to ingestion of water while losses were simultaneously proceeding at 0.08 to 0.20 per cent of B_0 per hour.

whole amount of food had been given (in parts A to E) at 4 p.m., the amount was now divided into portions proportional to the period of time before the next meal.

(a) The dog was kept in the stall for 6 hours, having received $\frac{3}{4}$ of the ration 18 hours previously. In four tests the remaining $\frac{1}{4}$ of the food was mixed with an equal weight of water and given at once. On the average the dog drank water in each subsequent half-hour, and body weight increased further for 2 hours and then gradually declined (*a*, fig. 3). During the decline water was occasionally drunk in each test, but not in amounts sufficient to compensate the simultaneous losses.

This experiment indicates that the periodicities of body weight were chiefly controlled by the periodicities with which food was spontaneously taken by the dog. Although some water was drunk without food, more water was taken at each draft when food had preceded it.

(b) In three tests, $\frac{1}{8}$ of the food was given dry at the beginning of the test, and $\frac{1}{8}$ of it 3 hours later. Body weight increased with each meal, partly because water was ingested soon after it. Thus body weight oscillated in shorter periods.

(c) Finally, in four tests, $\frac{1}{24}$ of the dry food was given each hour for 6 hours. Water was then drunk every hour, usually within 5 minutes after food was swallowed, and correspondingly body weight steadily increased (c, fig. 3). Somewhat more urine was produced in these tests.

Summary. It is evident that the pattern of food ingestion may dictate the pattern of water ingestion. The periods between water ingestion could be rendered so small that body weight increased steadily rather than in 6-hourly or in 24-hourly periods. Food ingestion obviated the rule that loss of weight preceded drinking. Rather, the proportion of solids to water within the body was modified by addition of the food instead of depletion of the water.

COMMENT. Drinking is a means of periodically restoring body water content. Previously (Adolph, 1939) it was found that the amounts drunk by the dog were equal to the amounts of water earlier lost from the body during water privation (which could be induced in one to several days while dry food was eaten). Now it is found that the smallest amount of water loss to which the dog usually responds is 0.5 per cent of the body weight. Not the time elapsed (which could be much reduced by heating the dog), but the shortage of water itself appears to set off ordinary drinking. And not the absolute amount of water but the decrement in the proportion of water to other bodily constituents (which could be varied by feeding the dog) seems to arouse the drinking.

These relations naturally result in the observed pattern of water ingestion of the dog. Ordinarily processes of anabolism and catabolism are producing slow changes in the proportions of bodily constituents. Whenever water is out of ratio by about −0.5 per cent of its usual content, a draft of it is taken, in approximately that amount. Usually less water is taken than has been lost since the last drink or meal.

This generalization pictures water content as continually in deficit. But since there is no base line from which to judge deficit other than the usual content of water in the body, it is merely for convenience of reference that water balance in these tests was considered to lie at the body weight of the dog that had just drunk water *ad libitum*. Obviously, too, body weight is but a rough criterion of water balance, since addition to the body of food and water in particular proportions increases the weight without destroying the immediate balance.

It was found that water was usually drunk a few minutes after dry food was eaten. Since the reverse rarely occurred, some support could be deduced for the notion that eating sets off drinking. If so, what releases the act of eating? With food continuously available, some cumulative influence periodically takes effect. To call that influence either hunger or appetite does not help to understand it. It is related to certain or all of the bodily deficits that gradually develop to some critical value before the trigger to eating is released.

The important point here is, however, that if the dog's five meals a day are stopped by deprivation of food, drinking that ordinarily follows each meal goes on just the same. The amounts drunk are reduced, but the same number of drafts are taken per day. Hence the pattern of drinking is demonstrated to exist independently of eating, though ordinarily the two work together in one sequence. The fact that some water is drunk during the deprivation of food

may indicate that water lost by the quiet dog is in excess of the body water liberated from its connection with catabolized constituents of the body. The constancy of urinary composition opposes the view that much water is drunk in excess of needs under any circumstances.

While the above rules held for the dogs studied in the laboratory, it is quite probable that deviations from these rules with respect to certain details will be found in other individuals, and particularly in individuals not conditioned to laboratory existence.

The sensitivity of the dog to the deficit of water which leads to drinking is such that on the average the dog drinks when 0.5 per cent of the body weight has been lost. Whatever sense organs or nerves are sensitive to want of water, and initiate sensations of thirst, themselves set in motion the response of drinking at this deficit of water. The result is an oscillation of water content of the body through sudden periodic intakes. But if food were supplied continuously at a rate equal to catabolism, the fluctuations to either side of the mean would be expected to amount to only 0.25 per cent of the body weight.

It is noted that the dog does not take its water in as continuously as the frog does. No terrestrial animal does so. It is appropriate to point out that intermittency of an activity allows successions of activities that cannot be carried on simultaneously. With respect to water, the dog is able to spend 99 per cent of its time away from water, providing that water can be reached at appropriate intervals. An animal as large as the dog is able, in absence of marked physical activity, to be away from water for 4 to 6 hours without change of more than 1 per cent in its water content. Hence body fluids and cells are kept approximately that constant in composition in spite of the intermittent character of intakes of water and food.

The picture that we derive from the above observations is that 1, the state of water balance is continually changing in relation to body weight, according as other metabolic processes are active; 2, change in rates of catabolism or sudden addition of food or solute to the body shifts the state of water balance accordingly; 3, the actual content oscillates about the virtual content at which water is balanced; 4, the period of this oscillation varies with rates of water exchange; but 5, the amplitude of this oscillation is ordinarily quite constant, through the fact that the dog drinks whenever the deficit of water is great enough to arouse an effective thirst. Many details remain to be added to this general statement.

SUMMARY

The conditions of water loss and of food gain were varied while spontaneous drinking was observed in dogs. The signal that initiates water drinking in ordinary life appears to be a deficit of water relative to other bodily components. Whenever the body is depleted of water by about 0.5 per cent of the body weight, water is drunk. The amount drunk is accurately proportioned to the body's water deficit at each draft, though no absorption of water has time to occur before drinking ceases. Body weight is only a rough criterion of water balance, even under restricted circumstances, since addition of food and loss of catabolic products complicate its relations.

REFERENCE

ADOLPH, E. F. This Journal 125: 75, 1939.

THE ACTION OF ADRENALINE, ACETYLCHOLINE AND POTASSIUM IN RELATION TO THE INNERVATION OF THE ISOLATED AURICLE OF THE SPINY DOGFISH (SQUALUS ACANTHIAS)[1]

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It seems fairly well established that the elasmobranch heart is not supplied with sympathetic nerve fibers. Lutz (1930a) has reviewed and confirmed the evidence for this view. Since the heart appears to be exclusively under vagus control, the isolated sinus-auricle preparation seemed a good one for the investigation of the action of adrenaline on myocardial tissue lacking sympathetic innervation. MacDonald (1925) and Lutz (1930b) have found adrenaline to have a vagus-like inhibitory effect on the heart beat of elasmobranchs, often followed by a stimulating action. However, this effect was with concentrations of adrenaline (1:25,000–1:100,000) unlikely to exist under physiological conditions in this animal's heart, particularly since, if there is no sympathetic supply, only the adrenaline in the circulating blood is available to affect the myocardium. In our reinvestigation of the effect of adrenaline we have used weaker and hence more nearly physiological concentrations. The study of the action of adrenaline has also led to observations on the actions of acetylcholine and potassium-excess.

METHODS. The sinus-auricle preparation of the spiny dogfish (Squalus acanthias) was suspended in a cylinder of 50 cc. capacity containing the physiological saline for elasmobranchs described by Lutz (1930b) and so arranged that contractions were recorded on a kymograph. A stream of oxygen was bubbled through the solution during the whole period of observation. Auricles continue to beat under these conditions for at least 24 hours.

Drugs were added in solution to the cup where they were quickly dispersed by the stirring action of the oxygen bubbles.

RESULTS. We were able to confirm the observation of MacDonald and Lutz that adrenaline in quite large concentrations (1:2000–1:100,000) will cause a temporary inhibition of the heart followed by a stimulation. In contrast to this finding, dilute solutions of adrenaline (1:1,000,000–1:200,000,000) caused only stimulation, which was recorded as an increase in the amplitude of the contractions without much change in rate. Neither of these effects of adrenaline could be blocked by atropine, which is evidence against the hypothesis of Lutz that the inhibitory action of adrenaline observed by him was due to stimulation of parasympathetic nerve endings.

In the absence of an inhibitor of choline-esterase (e.g., eserine), concentrations of acetylcholine as high as 1:5000 were required to produce any inhibitory effect. This is in marked contrast to the reaction of the auricles of frog and turtle hearts,

which under the same conditions show inhibitory effects when exposed to acetyl-
choline in concentrations a thousand times more dilute.   The implication of this
observation is that the choline esterase content of the tissue is quite high.   This
inference is strengthened by the observation that the auricles show a decreased
amplitude of contractions in an acetylcholine concentration of 1:100,000,000 after
treatment with prostigmine (1:500,000) (fig. 1), although much higher concen-
trations were required to cause complete suppression of the beat (fig. 2).   The
reaction of this auricle to acetylcholine is like that of other vertebrate auricles in
that small concentrations cause only a negative inotropic effect whereas greater
concentrations are required to cause negative chronotropic effects which eventu-
ally lead to complete cessation of contraction.

Potassium excess also suppresses the contractions of this auricle.   An excess of
30 mgm. per cent of KCl (in addition to the 60 mgm. per cent already present in
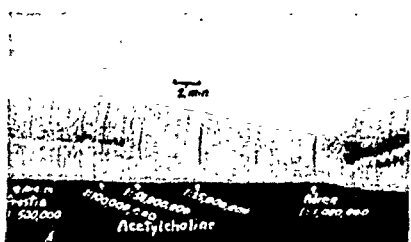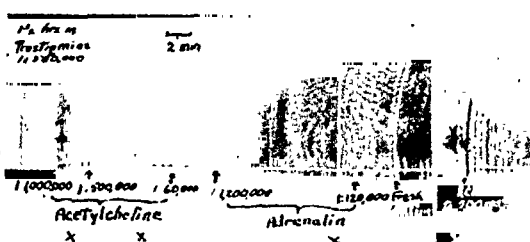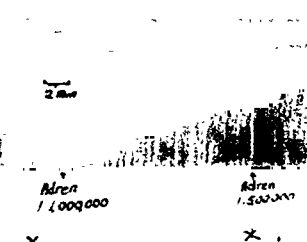
Fig. 1   Fig. 2   Fig. 3

Fig. 1. Effect of acetylcholine (1:100,000,000 to 1:25,000,000) after prostigmine
(1:500,000) on the isolated sinus-auricle of Squalus acanthias, and its reversal by adrenaline
(1:1,000,000).

Fig. 2. Effect of gradually increasing concentrations of acetylcholine (1:1,000,000 to
1:60,000) after prostigmine (1:500,000) on the isolated sinus-auricle of Squalus acanthias,
and its antagonism by adrenaline (1:1,200,000 and 1:120,000).   Sections of the original
record have been omitted at the points marked X.

Fig. 3. Effect of potassium excess (200 mgm. per cent KCl) on the isolated sinus-auricle
of Squalus acanthias and its antagonism by adrenaline (1:1,000,000 and 1:500,000).   Sec-
tions of the original record have been omitted at the points marked X.

the normal saline) caused a decrease in the amplitude of the contractions, and an
excess of KCl up to 200–300 mgm. per cent caused complete cessation of the beat
(fig. 3).   This action of potassium is not blocked by atropine and the evidence
seems conclusive that its effect is directly on the auricular myocardial cells, its
action being identical with that which has been demonstrated in the case of the
non-innervated turtle ventricle by Hiatt and Garrey (1943).

It is significant that weak concentrations of adrenaline antagonized not only
the neural inhibitory action of acetylcholine but also the myal depression pro-
duced by potassium-excess.   After the auricle has been inhibited to the point of
complete cessation of contraction by either of these agents, the addition of small
quantities of adrenaline causes a resumption of the beat and a recovery of the
original amplitude in the presence of the inhibiting or depressing substance
(figs. 2 and 3).   The corresponding antagonisms found in the auricles of frog or

turtle hearts do not approach the degree noted in these experiments on the dogfish heart.

DISCUSSION. If the elasmobranch heart lacks a sympathetic nerve supply, and this seems well established, then the site of action of adrenaline must be either on the vagus endings or directly on the myocardial cells. The fact that adrenaline effects, both augmentor and inhibitory, are unaffected by atropine would appear to indicate that the action is not on the vagus endings. Therefore the site of action of adrenaline, including the antagonism of the effects of acetylcholine and potassium-excess, must be directly on the myocardial cell. Hiatt and Garrey (1943) have shown that adrenaline has a slight augmentor action on the contractions of spontaneously beating strips of turtle ventricle, which they believe to be without autonomic innervation. The effect of adrenaline on the myocardium of the elasmobranch auricle is, however, much greater, and it would appear that the myocardial cells of the elasmobranch auricle have a special sensitivity to adrenaline.

The heart of the elasmobranch is apparently under constant vagal tone and since stimuli applied to almost any part of the body of an elasmobranch cause reflex cardiac inhibition (Lutz, 1930a), it is apparent the extreme sensitivity of the myocardium to adrenaline affords a mechanism of antagonism to acetylcholine even in the absence of sympathetic innervation of the heart. It has been shown by Lutz and Wyman (1927) that extracts of the chromophil bodies of this elasmobranch give characteristic adrenaline effects. Whether these glands are activated in an emergency is not yet known.

SUMMARY

1. The isolated sinus-auricle preparation of the elasmobranch (Squalus acanthias) which is apparently without sympathetic innervation, shows marked reactions to adrenaline.

2. The previously reported observation that adrenaline in high concentration causes a transient inhibition has been confirmed, but this effect is not blocked by atropine, so it is concluded that the action is not upon the vagus endings as has been suggested, but that it is directly upon the myocardial cells.

3. Adrenaline in low, more nearly physiological concentrations causes augmentation of the contractions without a change in rate. This effect also persists after atropine.

4. The dogfish auricle is much more resistant to the action of acetylcholine in the absence of an inhibitor of choline-esterase than the auricles of frog and turtle hearts, but after treatment with prostigmine the dogfish auricle reacts in the same manner and with approximately the same sensitivity as other vertebrate auricles to acetylcholine.

5. The inhibitory actions of acetylcholine and the depression of auricular beat due to potassium-excess are markedly antagonized by small concentrations of adrenaline.

6. A possible teleological value of the sensitivity of the myocardial cells to adrenaline is suggested.

The author wishes to express his gratitude to Dr. W. E. Garrey for his criticism during the writing of this paper.

REFERENCES

HIATT, E. P. AND W. E. GARREY.   In press, 1943.   This Journal.
LUTZ, B. R.   Biol. Bull. 59: 211, 1930a.   This Journal 94: 135, 1930b.
LUTZ, B. R. AND L. C. WYMAN.   J. Exper. Zool. 47: 295, 1927.
MACDONALD, A. D.   Quart. J. Exper. Physiol. 15: 69, 1925.

LOW HEART RATE IN THE NEWBORN RAT

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Curiosity about whether the heart rate of the one-day-old rat could be electrically recorded gave rise to the following series of experiments. It was found that heart rate could be recorded[1] and that, contrary to expectation, the rate of the day-old rat was considerably lower than that of the adult rat. The present series of experiments was planned to verify this surprising initial finding and to study the development of heart rate in the early stages of the rat's life.

Determination of heart rate in newborn rats. The heart beats were recorded by a Davis electroencephalograph; clip electrodes were attached ventrally and dorsally through the axis of the heart.

Heart rates were determined in 131 rats from 19 litters within 24 hours of birth. The rate per minute of each animal was computed from a 30-second strip of electroencephalograph tape. Fluctuation in heart rate was calculated by analyzing the 30-second period into five 6-second periods, and considering the maximum change between any two periods as the fluctuation. Data were analyzed for sex differences, and comparison with mature, 120-day-old rats was made.

RESULTS. *a.* A comparatively low heart rate was found in both male and female day-old animals. Sex differences in mean heart rate found in the tamed adults were not present in the newborn animals. (A slight but significant difference in body weight was found in favor of the males.) No sex differences in fluctuation of heart rate occur.

b. Significant differences in heart rate between male and female 1-day-old and 120-day-old rats were found. The 1-day-old rats had a much slower rate. The range of individual differences in mean heart rate (as indicated by "V", the coefficient of variability) decreases from the 1-day level to the 120-day level. Average fluctuation in heart rate increases significantly from the 1-day to the 120-day-old animals. Individual differences in average fluctuation (as indicated by "V") decrease from the 1-day to the 120-day-old animals.

c. Of the 131 rats, 99 (from 14 litters) had been tested immediately following birth, while 32 rats (from 5 litters) had been tested from 8 to 24 hours after birth. Data were re-analyzed to see whether heart rate differed between these two age groups.

Significant changes in heart rate occurred within the first 24 hours of life. No significant differences in mean fluctuation of heart rate were observed in the first 24 hours of life. Sex differences in mean heart rate were not present at birth or in the first 24 hours of life.

d. In the above analysis significant changes in heart rate were found in dif-

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[1] A check experiment in which the thoracic cavity was opened and the heart observed simultaneously with the recording ensures that it was the heart potential which was being recorded.

ferent rats during the first 24 hours of life. The aim of the present experiment was to follow the changes in heart rate in the same animals through the first 24 hours of life. To this end the following procedure was adopted: 12 animals,

TABLE 1

Mean heart rate and mean fluctuation in heart rate in a 30-second period of 131 rats during the first 24 hours of life

	MALES (N:66)	FEMALES (N:65)	CRITICAL RATIO*
Mean heart rate.....	174.6 ±44	168.5 ±42.7	0.8
Mean fluctuation of heart rate.	12.7 + 14.3	13.1 + 16.4	0.2

* This is an index of the reliability of a difference between two measures. A critical ratio of 3 or more indicates that the obtained difference is statistically significant, *i.e.*, the chances are greater than 999 in 1000 that a true difference exists.

TABLE 2

Comparison of mean heart rate and mean fluctuation in heart rate in 1-day-old and 120-day-old (tamed) ‡ rats

	1-DAY-OLD GROUP		120-DAY-OLD GROUP		CRITICAL RATIO COLUMNS (1) & (3)
	Mean (1)	"V"† (2)	Mean (3)	"V"† (4)	
Male rate......	175 ±44 (N = 66)	25.1 (23.8)*	433.5 ±22 (N = 16)	5	34.1
Fluctuation.....	12.7 ±14.3 (N = 66)	113 (116)*	22.3 ±8.2 (N = 16)	37	3.7
Female rate.....	168.5 ±43 (N = 65)	25.6 (25.5)*	476 ±24.6 (N = 11)	5	33.4
Fluctuation.....	13.1 ±16.4 (N = 65)	129 (133)*	19.5 ±3.6 (N = 11)	19	2.9

* Since it will be shown that heart rate varies significantly in the first 24 hours of life, the objection might be raised that considerable variability in the rate of the 1-day-old rats is due to the presence of different age groups (in terms of hours). To meet this objection, results were tabulated for 50 males and 49 females measured directly at birth. These results, not significantly different from those for the total group, are given in parentheses.

† Coefficient of variability.

‡ These animals had been habituated to the heart recording situation for a 2-week period before records were taken.

TABLE 3

Comparison of mean heart rate and fluctuations in heart rate of rats tested at birth and of rats tested 8 to 24 hours after birth

INDEX	GROUP TESTED AT BIRTH (N:99) (1)	GROUP TESTED 8-24 HOURS AFTER BIRTH (N:32) (2)	CRITICAL RATIO COLUMNS (1) & (2)
Mean heart rate...	161 ±40 (CR* sex diff: .6)	204.2 ±40 (CR* sex diff: .6)	5.3
Mean fluctuation in heart rate	14 ±18.4 (CR* sex diff: .2)	9.2 ±11.8 (CR* sex diff: .1)	1.6

* Critical ratio.

TABLE 4

Significance of differences in heart rate between 8-hour periods in the first 24 hours of life

PERIOD	MEAN HEART RATE (N:12)	CRITICAL RATIO OF SUCCESSIVE PERIODS
Birth....	172 ±14	
8 hrs........	211.3 ±16.8	6.3
16 hrs........	225.5 ±23	1.7
24 hrs........	257.3 ±23.6	3.4
Birth-24 hrs. ..		10.8

6 males and 6 females, from two litters, were tested immediately at birth and every 8 hours thereafter for the first 24 hours of life.

Results on the same animals confirm what had previously been found in

different animals, *i.e.*, significant changes in heart rate occur in the first 24 hours of life. The more detailed analysis in this experiment showed that within the first 8 hours of life significant changes are to be noted.

c. As a continuation of preceding experiments a study of changes in heart rate in the first 21 days of life was made. Seventeen rats, 10 males and 7 females, were tested daily at the same hour.

Mean daily heart rate over the 21-day period was computed for both sexes.

Increase in heart rate was greatest in the first 11 days of life. By this time the rates of both males and females exceed 400 and overlap at times with the range found for the tamed 120-day-old rats. From the 11th to the 21st day a plateau of little or no increase in heart rate occurs. Sex differences emerge more definitely from the 10th day of life on, the females having the higher rate. However, sex differences in heart rate in mature rats were found to exist only in tamed animals habituated to the recording system. Untamed mature animals showed no sex differences, nor did the tamed animals when recording was resumed after a 50 day interval. Evidence on hand would suggest an interpretation in terms of the differential reaction of the male and female heart under conditions of stress, the male rate increasing to a greater degree than the female and in this way reaching a level not significantly different from the females.

Discussion. In the foregoing experiments the most striking result concerns the comparatively low heart rate of the 1-day-old rat. The expectation had been that a rate of 600–800 beats per minute would be found at birth with a subsequent decrease to the adult level. Whether this low rate at birth is peculiar to the rat alone is not definitely known, for there has been little comparative work done in this respect. A few exploratory experiments carried out by the writers with newborn kittens and pigs showed the usual result, *i.e.*, a heart rate at birth higher than that of the adult. The mean heart rate of two newborn kittens was 168, while that of two adult cats was 131. In like fashion the mean heart rate of two newborn pigs was 227, and that of three adult pigs, 65. Similar results with other animals are reported by Dukes[2] and he concludes: "Young animals have a faster heart rate than mature animals . . . explained at least in part by their smaller size" (p. 115).

SUMMARY

1. One-day-old rats, when compared with 120-day-old (tamed) rats, were found to have: *a*, a significantly lower heart rate; *b*, less fluctuation in heart rate. Sex differences present in the tamed, mature animals were not present in the 1-day-old animals.

2. In the first 24 hours of life significant changes in heart rate were observed.

3. In a 21-day period of daily heart recordings, it was observed that:

a. Heart rate increased steadily in the first 11 days of life.

b. A plateau of no increase in rate prevails from the 11th to 21st days.

c. Sex differences manifest themselves with increasing clarity from the 10th day on.

[2] Dukes, H. H. The physiology of domestic animals. Comstock Press, Ithaca, N. Y., 1942, pp. 721.

DIFFERENTIAL EFFECTS OF STRETCH UPON THE STROKE VOLUMES OF THE RIGHT AND LEFT VENTRICLES

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In cinematographic studies on ventricular output Shuler, Ensor, Gunning, Moss and Johnson (1941) employed a modification of a technique developed in several laboratories by Takeuchi (1925), Strughold (1930), Landis, Hunt, Moe and Visscher (1940), and Burchell and Visscher (1941). Shuler and co-workers differentiated the effects of respiration on the output of the right and left ventricles and found that in each respiratory cycle the stroke volumes of the right and left ventricles varied independently. They reported an *increase* in the stroke volume of the *right* ventricle and a *decrease* in the stroke volume of the *left* ventricle during inspiration with the reverse changes occurring during expiration. These periodic independent fluctuations in output occurring in each ventricle were associated with corresponding independent fluctuations in the diastolic size of the two ventricles such that, when the diastolic size of the right ventricle increased in inspiration the stroke volume of that ventricle also increased, even though the diastolic size and stroke volume of the left ventricle simultaneously decreased.

In view of this evidence that each ventricle, independently, "obeys" the "Law of the Heart" (Patterson, Piper and Starling, 1914), which states that " . . . the energy set free at each contraction of the heart is a simple function of the length of the fibers composing its muscular walls," it was felt that further studies should be made on the independent operation of this law in the two ventricles.

METHODS. Dogs were anesthetized with sodium barbital. A portion of the sternum and ventral chest wall was removed and the pericardium was incised and sutured to the chest wall to cradle the heart. Numerous small circular cardboard markers were fixed to the heart clearly outlining measurable areas on the surface of each ventricle. Motion pictures were taken (at 32 or 48 frames per sec.) through a transparent window used to seal the opening in the chest wall, permitting the dog to breathe in the normal manner. In some experiments, the pictures were taken without sealing the chest wall, exposing the heart to atmospheric pressure.

Various procedures which change the diastolic size and, consequently, the force of the stroke were employed. These included: 1, increasing the venous return by rapid intravenous infusion of Ringer's solution; 2, increasing the peripheral systemic resistance by partially occluding the abdominal aorta, and 3, normal respiratory changes in intrathoracic pressure, pulmonary vascular capacity and ventricular filling.

Negative enlarged prints of many successive frames were made, and the out-

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lined area of each ventricle was measured in each frame.   Plotting these areas gave approximations to "volume curves" showing maximal diastolic size, minimal systolic size, and "stroke volume" for each beat of each ventricle, in arbitrary units. · For a number of heart beats, diastolic sizes of the right and left ventricles were plotted separately against the corresponding stroke volumes, and differences in the steepness of the slopes of the curves for right and left ventricles were interpreted as differences in the effectiveness of stretch of the muscle fibers of the two ventricles in inducing increases in stroke volume.

RESULTS.   Figure 1 shows typical results obtained in most instances in which changes in diastolic size and stroke volume were produced by means of intravenous infusion of Ringer's solution or compression of the aorta while the cardiac rate remained constant.   It is seen that the points for the right ventricle are less scattered and fall more nearly upon a line than those for the left ventricle.   Prob-
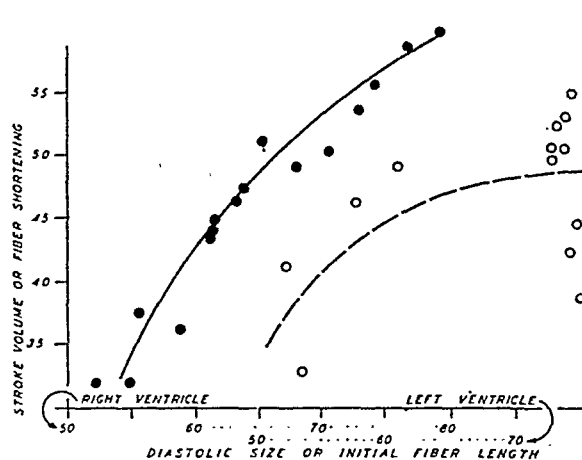
Fig. 1
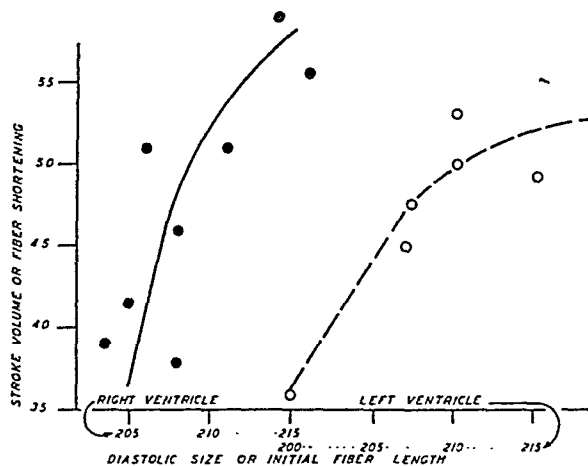
Fig. 2

Fig. 1. Effect of increase in diastolic size induced by intravenous saline infusion and aortic compression upon the stroke volume of the left and right ventricles determined separately.   Units are arbitrary.

Fig. 2. Effect of changes in diastolic size induced by normal respiration upon the stroke volume of left and right ventricles determined separately.   Units are arbitrary.

ably this indicates that the relationship between diastolic size and stroke volume is more direct in the right ventricle, i.e., it is influenced less by such other factors as changes in peripheral resistance or velocity of ejection than is the case in the left ventricle.

The greater steepness of the curve for the right ventricle seems to indicate that increased diastolic size (and stretch of the muscle fibers) is more effective in increasing the stroke volume of the right than of the left ventricle.

Figure 2 shows eight beats of the heart during a single respiratory cycle, in which the diastolic sizes and stroke volumes of the two ventricles fluctuate independently.   The results are essentially the same as in figure 1.   In the right ventricle, the points are again somewhat less scattered, and a given increment in fiber length seems to be more effective in increasing the systolic shortening of the fibers in the right than in the left ventricle.

In a few instances, the steepness of the slope of curves for the right and left ventricles was nearly the same, but the steepness of the left ventricular curve was never greater than that of the right ventricle in the same heart.

DISCUSSION. In attempting to account for these differences in response to stretch in the right and left ventricles, it must be borne in mind that in these experiments only *one* manifestation of increased work resulting from increased stretch is measured. For a complete analysis it is necessary to know not only the stroke volume but also the pressure (peripheral resistance) against which each ventricle acts as well as the factor for imparting velocity to the blood. For example, in aortic compression (one procedure providing data for fig. 1) the systemic peripheral resistance was certainly increased. Therefore, much of the extra energy set free by the stretched fibers of the left ventricle was employed to overcome the increased resistance. Consequently, less of the extra energy was available for increasing the stroke volume. On the other hand, compression of the aorta leads to a relatively slow and slight rise in pulmonary pressure (Johnson, Hamilton, Weinstein and Katz, 1937) so that a greater percentage of the extra work done after stretch of the fibers of the right ventricle was employed to produce increased stroke volume. However, when Ringer's solution is infused there is no such marked increase in arterial resistance in the systemic circulation as compared with that of the pulmonary system. Yet, essentially the same differences in the effects of stretch upon the two ventricles were seen.

The results plotted in figure 2 may also be partially explained by fluctuations in the resistance against which each ventricle pumps, in each respiratory cycle. When the diastolic length of the right ventricular fibers is greatest (i.e., in inspiration) the resistance against which that ventricle pumps is lowest, since pulmonary arterial pressure has been shown to fall in inspiration (Johnson, Hamilton, Weinstein and Katz, 1937). Therefore, the added force of contraction produces mainly an increased stroke. On the other hand, when the diastolic length of the left ventricular fibers is greatest (i.e., in expiration) the systemic arterial pressure is highest. Here the added force of contraction can be only partially employed to increase the stroke, since some of the added force must be used to pump against a higher pressure.

Visscher and Starling (1927) have shown that a poorly nourished heart must be stretched more than a well nourished one to induce the same increment of work. It seems unlikely that the left ventricle was regularly less well nourished than the right, in our experiments, since the systemic blood pressure remained at a good level throughout, and the fibers of both ventricles are supplied from the aorta. Furthermore, the left ventricular fibers are stretched the most during expiration at which time their blood supply should be greatest since the aortic pressure is highest at this time.

It seems probable that differences in the anatomical arrangement of the muscle fibers in the two ventricles may be mainly responsible for the results obtained. The muscle fibers are known to run a more nearly straight course in the right than in the left ventricle,[2] so that an increase in the area of an outlined portion

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[2] Jane Sands Robb: Personal communication.

of surface of a ventricle represents a greater actual increased initial length of a given fiber in the right ventricle than in the left.

It seems improbable that the properties of the muscle fibers of the two ventricles are inherently sufficiently different to account for the apparently greater effect of stretch upon the stroke of the right than of the left ventricle.

SUMMARY AND CONCLUSIONS

1. Using a cinematographic technique, the effect of varying the diastolic stretch of the cardiac musculature (i.e., initial fiber length) upon the subsequent systolic stroke (i.e., shortening in contraction) was determined in the left and right ventricles of dogs separately.

2. The relationship of initial diastolic fiber length to stroke volume was more direct in the right than in the left ventricle, presumably because the peripheral resistance or pressure factor in the work done by the heart fluctuates less in the pulmonary than in the systemic circuit.

3. A given increment in diastolic size of the right ventricle was found to be more effective in producing an increased stroke volume from that chamber than was the same increment in the left ventricle.

4. Differences in the nourishment or inherent properties of the left and right ventricles are probably not sufficiently great to account for these findings.

5. In some of the procedures employed (especially occlusion of the aorta), the smaller stroke volume increase of the left ventricle in response to stretch may be accounted for partially by a concommitant increase in peripheral systemic resistance. Under such conditions much of the extra energy is employed in overcoming the increased resistance, leaving less energy to increase the stroke volume. Since aortic occlusion does not elevate the pulmonary peripheral resistance appreciably, a larger proportion of the extra energy released by stretch of the right heart is employed in increasing the stroke volume.

6. The most important factor accounting for the apparently greater response of increased diastolic size in the right ventricle is probably the fact that the muscle fibers of the right ventricle pursue a straighter course than the fibers of the left ventricle, so that a given increase in diastolic size produces a greater actual stretch of the muscle fibers themselves in the right than in the left ventricle.

REFERENCES

BURCHELL, H. B. AND M. B. VISSCHER. Am. Heart J. 22: 794, 1941.
JOHNSON, V., W. F. HAMILTON, W. WEINSTEIN AND L. N. KATZ. This Journal 119: 344, 1937.
LANDIS, C., W. A. HUNT, G. K. MOE AND M. B. VISSCHER. This Journal 129: 400, 1940.
PATTERSON, S. W., H. PIPER AND E. H. STARLING. J. Physiol. 48: 465, 1914.
SHULER, R. H., C. ENSOR, R. L. GUNNING, W. G. MOSS AND V. JOHNSON. This Journal 137: 620, 1942.
STRUGHOLD, H. This Journal 94: 641, 1930.
TAKEUCHI, K. J. Physiol. 40: 208, 1925.
VISSCHER, M. B. AND E. H. STARLING. J. Physiol. 62: 243, 1927.

FACTORS INFLUENCING THE TEMPERATURE REGULATION OF BIRDS[1]

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In his recent paper on the development of body temperature of birds, Kendeigh (1939) recalls the early work of Edwards who in 1894 divided young birds into two categories, those with (precocial) and those without (altricial) temperature regulation at hatching. The chick has been suggested to be of the precocial group. Romanoff and Sochen (1936) and Romanoff (1941) demonstrated that although the chick embryo is poikilothermic during early development, it changes toward the homoiothermic state during incubation and reaches true homoiothermism four or five days after hatching.

The development and importance of shivering and panting in thermotaxis of birds is not well understood. Odum recently introduced a sensitive recording device for recording shivering and found (1942) that muscle tremors of shivering do not appear in small altricial birds until at least three days after hatching. Randall and Hiestand (1939) found that panting in the domestic fowl is roughly proportional to body temperature and that the panting center is relatively inactive at normal temperatures.

In this study experiments were devised to investigate the development of temperature control in the chick, the nature and significance of shivering and panting, the effect of relative humidity on the efficiency of panting in temperature control, and the upper lethal temperature.

Studies were conducted with barred-rock chickens (*Gallus domesticus*) and pigeons (*Columba livia*). Development of temperature control was studied upon chicks removed from the incubator two hours after hatching and at progressively older ages until after thermotaxic control was established. Body temperatures were recorded from thermometers placed to a constant depth in the cloaca, under the skin, and in some instances in the visceral regions. First muscle tremors of shivering were heard through a stethoscope placed upon the muscles of the body.

A temporator tube used in lowering body temperature in some experiments consisted of a large T-tube, one end of which was enlarged into a bulb. A smaller tube was inserted through the distal opening of the T-tube and water was pumped into the bulb and out by way of the side tube maintaining a constant circulation of water at any desired temperature. In other experiments the body temperature was decreased in an ice chamber, or increased by wrapping the body in an electric heating pad. In order to raise skin temperature while

maintaining a normal deep temperature, the body was wrapped in a heating pad while cold water was circulated through a rubber-lined metal collar placed around the neck.

In studying the effect of humidity in temperature control, the body temperature of pigeons was raised in a heating cabinet devised to allow changes in relative humidity.

EXPERIMENTAL RESULTS. *Development of body temperature control.* When young chicks (2 to 24 hrs. post hatching) were removed from the incubator their body temperatures closely approximated that of the incubator (38 to 39°C), and when exposed to laboratory temperatures of 26°C, their body temperatures rapdily fell as low as 31 to 32°C. Upon exposure to more severe environmental temperatures (ice chamber at 10°C) practically no resistance was shown to the
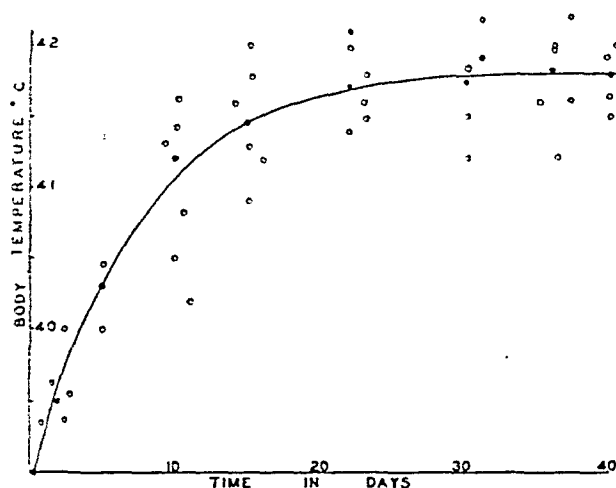
Fig. 1   Fig. 2

Fig. 1. The increase in body temperature of chicks from a few hours after hatching to that of chicks 40 days old. The curve represents average temperatures while the circles represent the range of variation. First two averages were taken with the birds in an environmental temperature of 38 to 39°C.; remainder at 20 to 26°C. This progressive development was studied in 40 birds.
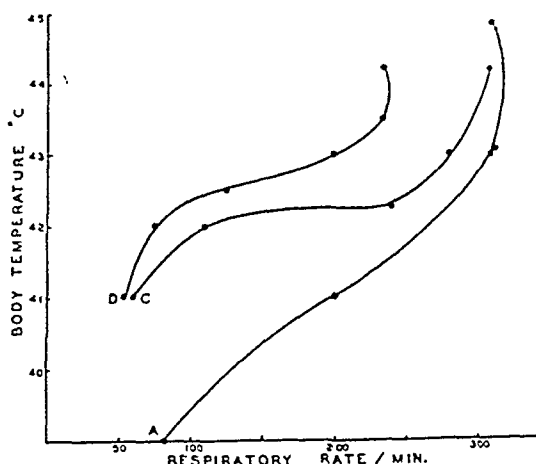
Fig. 2. Panting responses in young chicks as determined at different ages. Curve *A* is that of a two-day-old chick. *C* is that of the same chick fifteen days old and *D* is that of the chick twenty-eight days old.

chilling. Shivering was not pronounced and was often absent. The seven-day-old chick responded with vigorous shivering when exposed to the laboratory temperature and was completely capable of maintaining its body temperature within normal levels of 40.5 to 41°C. In fact, thermogenesis was sometimes sufficient to raise body temperature a few tenths of a degree. Exposure to temperatures below 20° still caused a rapid fall in temperature however and protection against such environmental temperatures did not develop until the down feathers were well replaced by adult plumage.

Two days after hatching the body temperature was above that of the incubator and a continued average rise was recorded until about 22 days after hatching (fig. 1). The most rapid increase occurred in the first ten days and there was but little change except for the usual diurnal variations after 15 days.

Newly hatched birds were found to be fully capable of responding to increased body temperatures by polypneic breathing which quickly assumed the characteristics of panting, but the panting was initiated at a lower level than in adults (table 1).

The gradual rise of the panting threshold parallels the rise of normal body temperature, but since it does not rise as rapidly, there results a progressive decrease in the thermal tolerance. Thus the thermal tolerance gradually ap-

TABLE 1

Panting responses of a typical chick during development from 1 day to 42 days of age

This chick was chosen as typical of 20 birds

AGE	CLOACAL TEMPERATURE	PANTING THRESHOLD	THERMAL TOLERANCE
Days	°C.		
1	38.0	41.0	3.0
5	40.0	42.0	2.0
10	40.8	42.1	1.4
15	41.4	42.5	1.1
22	41.7	42.8	1.1
42	41.7	43.0	1.3

TABLE 2

A condensed protocol chosen as typical of responses of the week-old chick to decreasing body temperature

Thirty birds were used

TIME	CLOACAL TEMPERATURE	SKIN TEMPERATURE	HEART RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	REMARKS
Minutes	°C.	°C.			
0	42.0	41.8	300	50	
2	41.9	41.0	360	70	First tremors
10	41.3	40.0	360	80	Continuous shivering
30	38.0	35.0	320	80	
52	35.0	31.0	270	76	
75	31.0	28.0	200	52	
110	26.0	23.0	108	40	Shivering decreasing
130	23.0	20.0	52	24	
150	20.0	18.0	20	14	Shivering ceased
180	15.0	13.0	12	0	

proaches that of the adult, the panting threshold becomes sharper and the efficiency of the panting mechanism increases (fig. 2).

Shivering and falling body temperature. The first muscle tremors preceding shivering in the seven-day chick (in ice chamber at 10°C) were noted after a fall of 1 to 3°C skin temperature with little or no change in cloacal temperature. Vasoconstriction and periodic muscle tremors were quickly followed by continuous shivering and in all instances were accompanied by faster breathing and heart rates (table 2). With progressively falling body temperatures shivering began to decrease in intensity at about 26°C and ceased entirely at about 20°C.

Respiration failed at 15°C and although the heart became very weak, biopsy showed that it continued to beat at temperatures as low as 8.5°C. Administration of 100 per cent oxygen into the trachea and out by way of a punctured abdominal airsac maintained a respiratory rhythm (6 per min.) at a temperature of 10°C indicating that hypothermic death is primarily caused by anoxic paralysis of the respiratory mechanism. Complete recovery was brought about if body temperature was returned to normal in a heating pad providing cloacal temperature was not reduced previously below 15°C.

To further investigate the existence of reflex and central shivering, a temporator tube was placed in the cloaca and body temperature was lowered without subjecting the skin to sudden changes in temperature. In contrast to the above experiments, shivering first appeared only after a fall of 1.2°C cloacal temperature while the skin temperature was held relatively constant. It therefore appears that shivering may be produced reflexly by stimulation of cold receptors in the skin or centrally by cooling effects of the blood bathing thermogenic centers.

Panting and rising body temperature. In contrast to experiments upon mammals, reflex panting of birds has not been demonstrated. An experiment was therefore devised whereby skin temperature was raised while at the same time the carotid blood was maintained at or below normal temperature. This was accomplished by placing the bird in a heating pad while the metal collar with circulating cold water was in place around the neck.

With this technique it was possible to maintain the deep body temperature at a constant level for two hours even though the skin temperature was raised nearly four degrees. This indicates the cooling effect of the collar was sufficient to chill the blood enough to offset the warming of the body through the skin. Breathing remained normal in rate and amplitude. The temperature of the esophagus under the collar was 37.6° while that of the skin under the heating pad was 45.0°C. Upon removal of the collar, with heating pad still in place, the breathing rate increased instantly from 34 to 150 per minute (table 3). Thus even though the temperature of the skin was held at or above panting levels for 2 hours, panting did not occur until the thermoregulatory centers were warmed to panting levels by the circulating blood. This indicates a discrete central control of panting in the fowl.

Effects of varying humidity upon panting. Since birds have no sweat glands and since feathers prevent much evaporation from the skin, the burden of heat loss must fall upon respiration. It is therefore important to know the effects of varying humidity upon the ability of birds to adequately control body temperature (table 4). The panting threshold was not markedly changed when both environmental temperature and relative humidity were increased, nor was thermal tolerance significantly altered. The bird's ability to prevent a rapid rise in body temperature was greatly inhibited, however. The time required to reach the panting threshold was decreased and the efficiency of the panting mechanism was decidedly less than normal, due to decreased evaporation of water.

The upper lethal temperature of birds. Available literature has revealed no definite information concerning the physiological effects of prolonged and acute hyperthermia. After reaching the polypneic stage of panting, with continuous hyperthermia, respiratory failure was one of the first indications of physiological breakdown. This followed a preliminary slowing from the maximum breathing rate and occurred at a temperature above 45.5°C. As cloacal temperature approached 47.0°, rising more rapidly after failure of the panting mechanism, the heart began to decrease in rate. Breathing was reduced to a few dyspneic

TABLE 3

A condensed protocol showing respiratory responses produced by rising skin temperatures while blood flowing to the brain is cooled

TIME	CLOACAL TEMPERATURE	SKIN TEMPERATURE	HEATING PAD	COLLAR	RESPIRATORY RATE PER MINUTE
Minutes	°C.	°C.	°C.	°C.	
0	41.6	41.4	Normal	Normal	45
10	41.4	43.0	56	10	45
40	41.7	43.0	56	10	36
80	41.8	44.4	60	9	32
120	42.0	45.0	60	7	34
Cold collar removed from neck					
123	42.1	45.0	60	Off	150
140	42.6	45.4	56	Off	200

TABLE 4

Showing typical effects of increasing humidity and temperature upon panting responses of adult pigeons

Twenty experiments were conducted upon 5 birds

STARTING TEMPERATURE	AVERAGE RELATIVE HUMIDITY	PANTING THRESHOLD	MINUTES TO REACH THRESHOLD	THERMAL TOLERANCE
°C.				
42.5	20 ± 5%	43.1	37	0.6°
42.6	34 ± 5%	43.1	20	0.5
42.5	48 ± 5%	43.2	15	0.7
41.5	54 ± 5%	42.5	10	1.0
42.7	75 ± 5%	43.0	5	0.3

gasps interspaced by apnea. Death was always immediately preceded by increasing tonus of skeletal muscles which reached convulsive stages just prior to complete respiratory failure. The convulsions were characterized by a "stretching" reaction similar to those noted in asphyxial death. These convulsions then were replaced by decreasing clonic contractions; the heart became very irregular and weak. Immediate autopsy showed that the heart had completely stopped except for signs of flutter or fibrillation. All of these later changes were noted within a period of one to three minutes before death.

In young birds the lethal temperature varied more than in adults, the range of variation being between 46.0 and 47.8°. However, an average of 47.0° compares closely with the less variable average found in adults. Administration of oxygen always maintained respiratory and heart functions to somewhat higher temperatures, and the lethal temperature was in all instances higher than in those experiments without oxygen therapy. Temperatures as high as 48.8° were recorded before sudden muscle atony, arrhythmia, and final heart failure ended in death.

DISCUSSION. As pointed out by Baldwin and Kendeigh (1932), the mass or size of the body of birds increases proportionately faster than does surface area. Thus as more protoplasmic tissue is involved in heat production with comparatively little change in area for dissipation of heat, the normal body temperature must rise.

Evidence indicates that chemical mechanisms of temperature control are developed during incubation and in the first few days of the post-hatching period. It has been noted by others that the period of maximal basal heat production and growth occurs fifteen days after hatching, a period which corresponds closely with the attainment of adult body temperature levels as shown in figure 1. Further control then depends upon the formation of an external insulation of feathers.

Since the heat regulatory mechanism of young chicks is functional within a few hours after hatching, it appears that the chick is potentially homoiothermic at hatching, but upon exposure to temperature extremes, may revert toward the poikilothermic state of its embryological development.

Shivering in birds appears to consist of two separate and distinct physiological components. The first is reflex shivering initiated by impulses arising in skin receptors (possibly following an increased metabolism due to hormonal control). Reflexly increased breathing rates represent increased muscular contraction designed to raise metabolic production of heat. Secondly, since shivering may be produced while deep body temperature is decreased but skin temperature is held constant, a centrally initiated shivering is indicated. General depression of all oxidative functions is probably involved in cessation of shivering at temperatures below 20°C.

The lack of oxygen seems the significant cause of death at low temperatures. Drastic slowing of the heart results in failure to circulate sufficient oxygen to the vital centers of the brain and breathing fails. The remarkable resistance of the "warm-blooded" heart of the young chick is demonstrated in the intrinsic beat maintained at temperatures below 10.0°C. Such endurance compares favorably with poikilothermic hearts.

Water loss occurs mainly in the mouth and pharynx as air is passed rapidly back and forth over these areas. The temperature of inspired air, together with humidity, controls to a large extent the amount of heat which can be lost by panting. As the temperature rises, relative humidity of environmental air decreases, and as a result, an increased amount of evaporation from expired air is possible. If, however, the relative humidity is raised simultaneously, evapora-

tion necessarily varies in an opposite direction, i.e., less water evaporates and less heat is lost.

It is of interest to note that the lethal temperature of birds, which is two to three degrees higher than that of mammals, can be correlated with the normal body temperature which is three to five degrees higher. (*This information emphasizes the important physiological and ecological fact that homoiothermic animals are living in the upper limits of their tolerable temperature range.*)

The exceedingly rapid rate of panting in birds has been shown to be due to overheating of a panting center located in the brainstem above the respiratory centers in the medulla (von Saalfeld, 1936). When the body temperature exceeds the threshold of this center polypnea is initiated by the respiratory centers as influenced by the higher center. With continuous hyperthermia, the failure of respiration just prior to death may indicate one, or a combination of, the following events: the panting center ceases firing, the impulses are blocked en route to the medullary centers, or the latter centers are rendered insensitive to the descending impulses. Failure of all breathing movements soon after the preliminary slowing may be suggestive of the last possibility. It has recently been found in our laboratories that birds are greatly dependent upon the integrity of the vagi for functional panting, and it is thus possible that vagal failure may be involved.

Acute anoxia is certainly important in the causation of death at high temperatures and the usual sequence of events would appear to be: paralysis of the respiratory centers and consequent respiratory failure (possibly following damage to thermotaxic centers), heart and circulatory arrest due to asphyxia, and finally cessation of all oxidative functions due to tissue anoxia.

SUMMARY

1. Normal body temperature of the barred-rock chick increases from a temperature identical with that of its environment (incubator at 38 to 39°C) to about 41° ten days after hatching, after which time it approaches and remains within the limits of the dirunal variation of the adult.

2. The chick is capable of panting and shivering at or shortly after hatching, but neither mechanism is efficient in maintaining body temperature until several days of post-hatching development.

3. Development of temperature regulation is correlated with increasing metabolism and body temperature, stabilization of central thermotaxic control, and the transition from down feathers to the adult type of plumage.

4. Reflex shivering may be evoked through skin receptors and shivering may also be centrally initiated by cooling of brain centers. Reflex panting could not be demonstrated in birds; only a discrete central control of panting is believed to exist.

5. When exposed to cold environmental temperatures, periodic muscle tremors, vasoconstriction, and increased breathing and heart rates are observed. With continued hypothermia, shivering becomes continuous until, like heart and breathing rates, it progressively declines. Shivering ceases at approxi-

mately 20°C, breathing stops at about 15°C, but the heart may continue to beat at temperatures below 10°C. Respiratory and heart functions can be maintained at even lower temperatures with oxygen.

6. Neither the panting threshold nor thermal tolerance of pigeons was significantly altered at high temperatures and humidity, but severe hyperthermia developed rapidly.

7. Although some variation exists in the lethal temperature of immature birds, an average lethal temperature for fowls may be taken as 47.0°C. The sequence of physiological events leading up to high temperature death is presented and discussed.

REFERENCES

BALDWIN, S. P. AND S. C. KENDEIGH. Sci. Pub. Cleveland Mus. Nat. Hist. **3:** 1, 1932.
KENDEIGH, S. C. J. Exper. Zool. **82:** 419, 1939.
ODUM, E. P. This Journal **136:** 618, 1942.
RANDALL, W. C. AND W. A. HIESTAND. This Journal **127:** 761, 1939.
ROMANOFF, A. AND M. SOCHEN. Anat. Rec. **65:** 59, 1936.
ROMANOFF, A. Science **94:** 218, 1941.
VON SAALFELD, E. F. Ztschr. Vergl. Physiol. **23:** 727, 1936.

RESPIRATORY AND CIRCULATORY RESPONSES TO ACUTE METHEMOGLOBINEMIA PRODUCED BY ANILINE[1]

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Methemoglobinemia may be produced by a considerable number of drugs and toxic agents (1, 2). The symptoms in methemoglobinemia (3, 4) are generally similar to those caused by an equivalent degree of CO-hemoglobinemia, and this is to be expected since the mechanisms by which they produce anoxia are similar. The various methemoglobin forming agents differ in the amounts of methemoglobin they produce in various species, and also they differ qualitatively in their other pharmacological actions. Since the symptoms in methemoglobinemia have often been attributed to the direct toxic actions of the methemoglobin producing agent, the mechanism and effects of methemoglobin anoxia require further investigation in the light of current concepts of the factors controlling respiration and circulation.

In the course of investigations on a series of aromatic amino derivatives the acute actions of aniline were studied in some detail (5). It was found that in the dog the production of methemoglobinemia was the most important action and apparently accounted for most of the resulting symptoms. Small doses produced a marked and fairly prolonged methemoglobinemia without significantly altering the blood pressure. These investigations suggested that aniline could be employed as a methemoglobin producing agent for a study of the effects of methemoglobinemia on respiration and circulation.

The fact that methemoglobinemia is accompanied by a corresponding decrease in the O_2 capacity of the blood is well recognized. However, no quantitative data could be found correlating respiration during methemoglobinemia with pH and gas contents of arterial blood. Similarly no data on cardiac output appear to be available, nor data on A-V O_2 differences and estimates of venous O_2 saturation.

Therefore, a series of experiments have been carried out on unanesthetized dogs to determine the effect of methemoglobinemia on pulmonary ventilation, cardiac output, and the pH and gas contents of arterial and venous blood.

METHODS. The experiments were performed on unanesthetized trained dogs after 16 to 20 hours' fasting. Each observation consisted of measuring ventilation and O_2 consumption and of obtaining samples of arterial and mixed venous blood. A control determination was made in the morning, aniline was then administered, and the experimental observation carried out $3\frac{1}{2}$ to 9 hours later. In some instances only control or experimental observations were made in a single day.

Methemoglobinemia was produced by the oral administration of aniline, 50 mgm. per kgm., as a 2 per cent solution. We have shown that in dogs this dosage produces a maximal methemoglobinemia of 50 to 65 per cent in 4 hours, which persists for approximately another 4 hours and then progressively diminishes (5). At least two weeks intervened between successive administrations of aniline.

Strict conditions for basal metabolism were maintained; the preliminary rest period was never less than 30 minutes and was usually 45 to 60 minutes. A mask was fitted over the dog's muzzle and connected through rubber valves to outside air. The expired air was collected in a 60 liter spirometer and analyzed with the Haldane-Henderson-Bailey apparatus. The volume of expired air was reduced to 0°C., 760 mm. Hg, and dryness for calculating metabolism, and to 37°C., prevailing barometric pressure and complete saturation with water for pulmonary ventilation. The heart rate was counted from the femoral pulse during the 4 to 8 minutes of expired air collection which immediately preceded the blood sampling. Mixed venous blood was drawn from the right heart, and arterial from the femoral artery. Cardiac output was calculated utilizing the Fick principle (see (6) for details of the method). Rectal temperature was noted in each experiment. Room temperature during the entire series varied from 21 to 26°C. However, the change during an individual experiment was never more than 1.5°C.

Determinations were made of arterial and venous blood pH (7); CO_2 and O_2 contents (8), and cell volume (9), and of arterial O_2 capacity (after equilibration with room air at room temperature (8)), total hemoglobin (THb) and methemoglobin (MHb) (10). Corrections were made for physically dissolved O_2 (11). The blood samples were collected in oiled syringes, and mixed under oil with heparin in iced containers. Samples for gas analyses and THb and MHb were immediately transferred to mercury tonometers containing dried fluoride and analyzed within an hour. The heparinized samples were used for the pH and cell volume determinations.

The values for MHb are expressed as percentages of the THb. The percentage arterial O_2 saturation was calculated from the O_2 content and capacity using the formula $HbO_2(100)/Hb + HbO_2$. Venous O_2 saturation was calculated similarly using the venous O_2 content and arterial O_2 capacity.

RESULTS. Twenty-six experiments were carried out on 3 dogs. There were 14 control determinations, and 12 observations following aniline administration with MHb concentrations varying from 48 to 68 per cent. The results are summarized in figures 1 and 2, and four representative experiments are shown in table 1.

The most important change in arterial blood was a reduction in O_2 capacity. The arterial O_2 saturation of the active hemoglobin remained practically unchanged since the decrease in O_2 content paralleled O_2 capacity. There were no significant changes in THb and cell volume. Arterial pH was not greatly altered. In dog 1 some tendency to a decrease was observed, but in dog 2 the tendency was toward an increase which was really significant in only one experi-
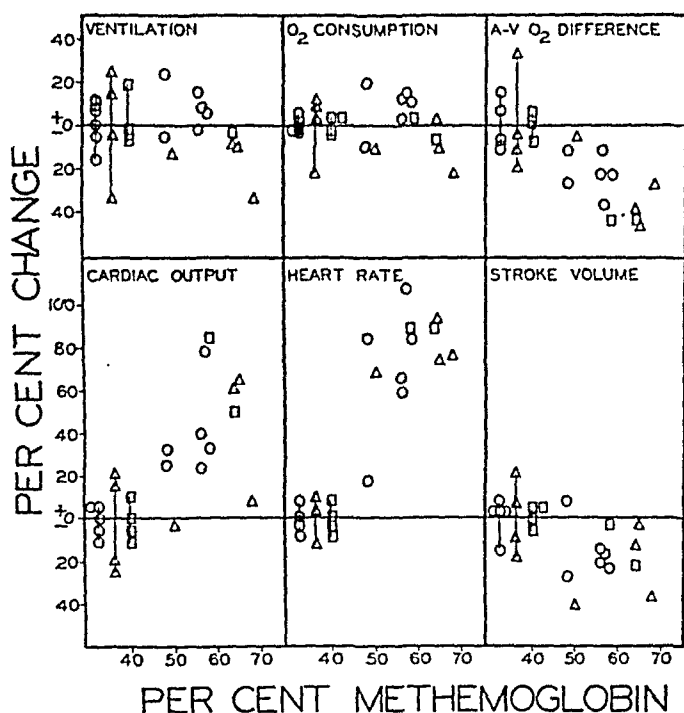
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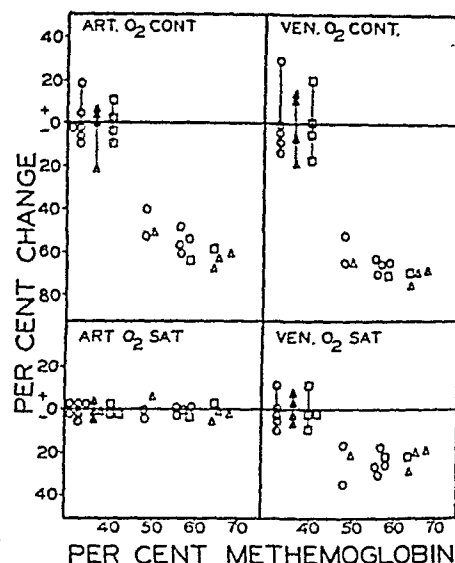
Fig. 2

Fig. 1. Effects of methemoglobinemia on ventilation and circulation. The vertically connected points show the deviation from the average control value for each dog. The changes during methemoglobinemia are calculated from the control of each experiment if available, otherwise from the average control value for each dog. Dog 1 $-\bigcirc$, 2 $-\triangle$, 3 $-\square$.

Fig. 2. Effects of methemoglobinemia on arterial and venous blood. The conventions are the same as in figure 1.

TABLE 1

DOG NO.	VENTI-LATION	O₂ USED	A-V O₂ DIFF.	CARDIAC OUTPUT	HEART RATE	ARTERIAL BLOOD				VENOUS O₂ SAT.	METH-EMO-GLOBIN
						pH	CO₂ Cont.	O₂ Cont.	O₂ Sat.		
	l./min.	*cc./min.*	*cc.*	*l./min.*			*vol. per cent*	*vol. per cent*	*per cent*	*per cent*	*per cent*
1	4.7	84	46	1.8	55	7.31	40.0	18.0	99	74	0
	4.6	86	35	2.5	91	7.29	39.6	7.5	99	53	56
2	5.7	94	40	2.3	71	7.34	40.2	19.3	99	75	0
	3.8	73	29	2.5	125	7.37	43.4	7.5	97	60	68
	5.2	93	60	1.6	66	7.34	44.6	18.6	98	67	0
	4.7	82	32	2.6	114	7.35	44.6	7.1	97	53	65
3	5.4	93	53	1.8	56	7.33	40.4	15.5	97	64	0
	5.2	87	32	2.7	107	7.35	43.5	6.4	99	50	64

ment. Arterial total CO₂ content was variable, and in some instances the change could not be correlated with the changes in pH and ventilation.

Ventilation remained unchanged in most of these experiments; a slight decrease was observed more frequently than a slight increase. Also in 10 other experiments under similar basal conditions, no hyperpnea was noted. In some of these latter experiments the effect of inhalation of pure O_2 for 5 minutes was tested. Breathing pure O_2 produced no significant decrease in ventilation as compared to the controls.

The O_2 consumption did not vary more than $\pm$ 15 per cent although in 2 individual experiments it was slightly greater. Rectal temperature remained unchanged, or decreased slightly. Only in dog 2 was a decreased rectal temperature correlated with a decreased O_2 consumption and ventilation.

Venous O_2 content was markedly reduced. This decrease did not exactly parallel the decrease in O_2 capacity (or the increase in MHb) because in most instances there was a decrease in A-V O_2 difference. Venous O_2 saturation was significantly decreased in most cases.

The A-V O_2 difference was usually decreased without a great change in O_2 consumption. Consequently in all experiments except two an increase in cardiac output was observed. The acceleration in pulse rate was frequently greater than the increase in cardiac output and in those cases the stroke volume was decreased.

DISCUSSION. The foregoing results were obtained on trained unanesthetized dogs and are therefore free from the effects of anesthesia. We wish to emphasize that these are relatively acute experiments with a maximum methemoglobinemia of 48 to 68 per cent produced by aniline. The mechanism of the MHb formation has been recently reviewed (5, 12), and experimental evidence of the respiratory and circulatory effects of aniline has been presented (5). From this evidence (5) we are convinced that the effects reported in this paper resulting from a small oral dose of aniline are due predominantly to MHb anoxia. At present we are not aware of any agent entirely free from other pharmacological actions which could have been employed to produce the methemoglobinemia.

Respiration does not appear to be stimulated by methemoglobinemia *per se*. It is now well established that respiratory stimulation in anoxemia is dependent upon stimulation of the carotid and aortic chemoreceptors (13). The effective stimulus for these receptors is a diminished arterial O_2 tension. A decreased arterial O_2 content without a sufficient decrease in pO_2 is ineffective as shown by Comroe and Schmidt (13b) by perfusing the carotid body with blood containing HbCO at normal pO_2. Anoxia has only a depressant effect on the respiratory center itself (13c, d). In our experiments we have not observed a significant decrease in arterial O_2 saturation and there is no reason to believe that arterial pO_2 is markedly altered. Therefore, no respiratory stimulation would be expected. On the other hand, some respiratory depression might have been anticipated. Our experiments produce no conclusive evidence on the latter point.

Definite circulatory stimulation was evidenced by the increased cardiac output. This was associated with a marked acceleration in pulse rate and some tendency to a reduction of stroke volume. Although blood pressures are not reported in this paper, observations on other animals under similar conditions have shown no great change.

The increased cardiac output clearly represents a compensatory response of the circulatory system. It is evident that a decreased arterial O_2 saturation cannot be evoked as an explanation. Some diminution of tissue pO_2 undoubtedly occurred since the venous O_2 saturation falls despite the increased cardiac output. The mechanism by which tissue anoxia (in the absence of a decreased arterial O_2 saturation) causes an increased cardiac output is obscure. Nevertheless, an increased cardiac output has been reported in acute carbon monoxide poisoning of dogs and man (14), and in anemia of man (15, 16) and of the dog (17). The rôles played by the increased pulse rate and vasomotor reactions in causing an increased cardiac output in this type of anoxia remain to be investigated.

MHb anoxia is due to a reduction in the O_2 carrying capacity of the blood. This results in a decrease in venous O_2 saturation, and consequently in venous pO_2. Darling and Roughton (12) have reported that MHb causes a definite shift to the left of the normal O_2 dissocation curve of hemoglobin. This shift will therefore cause a considerably greater decrease in venous pO_2 than is indicated by the venous O_2 saturation. For example, in an experiment on dog 2 with 68 per cent MHb, the venous O_2 saturation is 60 per cent. Approximation from the normal O_2 dissociation curve (18) gives a pO_2 of 33 mm. Hg, but from the MHb O_2 dissociation curve (12) only 19 mm. Hg. We have not shown estimates of pO_2 in this paper since the data of Darling and Roughton on dog blood are insufficient to permit this.

We have previously reported that MHb concentrations of 70 to 75 per cent in dogs caused unconsciousness, and motor release phenomena indicating cerebral depression (5). In the experiments here reported no gross neurological signs were evident other than slight lethargy, except in dog 3 which showed some neuromuscular weakness.

Our results on MHb anoxia are qualitatively similar to those reported for carbon monoxide poisoning by Chiodi, Dill, Consolazio and Horvath (14). In CO-hemoglobin anoxia the circulatory effects were apparent at somewhat lower concentrations of inactive hemoglobin, as might be expected from the more marked effect of HbCO on the O_2 dissociation curve. Since carbon monoxide is free from the objections which may be made against agents producing MHb, these observations would appear to lend support to our interpretation of the effects of methemoglobinemia.

SUMMARY

1. The effects of MHb concentrations of 48 to 68 per cent, produced by an oral dose of 50 mgm. per kgm. of aniline on trained unanesthetized dogs are described.

2. No respiratory stimulation was observed.

3. Cardiac output and pulse rate were significantly increased.

4. Arterial O_2 content was reduced in direct proportion to the MHb, but arterial O_2 saturation remained unchanged.

5. Venous O_2 saturation decreased despite the increased cardiac output.

6. The effects of methemoglobinemia on arterial and venous O_2 tension are discussed.

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We also wish to thank John Romano for technical assistance.

REFERENCES

(1) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. 1, Williams & Wilkins Co., Baltimore, 1931.
(2) WENDEL, W. B. Modern medical therapy in general practice. Williams & Wilkins Co., Baltimore, p. 3015, 1940.
(3) HALDANE, J., M. B. MAKGILL AND MAVROGORDATO. J. Physiol. 21: 160, 1897.
(4) HAMBLIN, D. O. AND A. F. MANGELSDORFF. J. Ind. Hyg. and Tox. 20: 523, 1938.
(5) CLARK, B. B., E. J. VAN LOON AND R. W. MORRISSEY. J. Ind. Hyg. and Tox. 25: 14, 1943.
(6) MARSHALL, E. K. This Journal 77: 459, 1926.
(7) DILL, D. B., C. DALY AND W. H. FORBES. J. Biol. Chem. 117: 569, 1937.
(8) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. 2, Williams & Wilkins Co., Baltimore, 1932.
(9) WINTROBE, M. M. Am. J. Med. Sci. 185: 58, 1933.
(10) EVELYN, K. A. J. Biol. Chem. 115: 63, 1936.
    EVELYN, K. A. AND H. T. MALLOY. J. Biol. Chem. 126: 655, 1938.
(11) SENDROY, J., R. T. DILLION AND D. D. VAN SLYKE. J. Biol. Chem. 105: 597, 1934.
(12) DARLING, R. C. AND F. J. W. ROUGHTON. This Journal 137: 56, 1942.
(13a) SCHMIDT, C. F. AND J. H. COMROE. Physiol. Rev. 20: 115, 1940.
(13b) COMROE, J. H. AND C. F. SCHMIDT. This Journal 121: 75, 1938.
(13c) DUMKE, P. R., C. F. SCHMIDT AND H. P. CHIODI. This Journal 133: 1, 1941.
(13d) WATT, J. G., P. R. DUMKE AND J. H. COMROE. Federation Proc. 1: 90, 1942.
(14) CHIODI, H., D. B. DILL, F. CONSOLAZIO AND S. M. HORVATH. This Journal 134: 683, 1941.
(15) NIELSEN, H. E. Acta. Med. Scand. 81: 571, 1934.
(16) LILJESTRAND, G. AND N. STENSTRÖM. Acta. Med. Scand. 63: 130, 1925–26.
(17) BLALOCK, A. AND T. R. HARRISON. This Journal 80: 157, 1927.
(18) DILL, D. B., H. T. EDWARDS, N. FLORKIN AND R. W. CAMPBELL. J. Biol. Chem. 95: 143, 1932.

DIETARY "SELF-SELECTION" AND APPETITES OF UNTREATED AND TREATED ADRENALECTOMIZED RATS[1]

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Richter (1–4) has described certain beneficial changes in appetite and food, water, and electrolyte selection in self-selection dietary experiments with adrenalectomized rats. He has observed decreased sodium taste thresholds with increased sodium intake (5–9), certain changes in water intake (3, 5, 6, 8, 9), and lastly, a decreased carbohydrate intake (6, 7), when adrenalectomized rats were allowed to select sodium solutions and sugar from separate feeders and drinkers. These results and those obtained in other studies by Richter, have been used to support certain claims purporting to partially elucidate "biological drives," "behavior," "adaptability," "effort to maintain a constant internal environment," and so forth.

It is obvious that if Richter's claims could be confirmed, such phenomena would be of fundamental biological importance. When Richter's work (6, 7) appeared on sugar and salt appetites of self-selecting adrenalectomized rats, we had been working along somewhat similar lines, and since we used a somewhat different technique and obtained different results, it was thought of interest to present these findings at the present time.

METHODS. Rats of uniform weight and sex, of a strain from which the Wistar colony originated (10), were divided into two groups of three to ten rats each, and placed into two cages, in which the various components of an adequate synthetic diet were separately offered in different feeders and drinkers. The cages were made of one-half inch hardware screen and were of the approximate dimensions, 18 inches x 13 inches x 8 inches deep. A refuse drawer was built under each cage, and clamp holders for the various drinkers were soldered to the backs of the cages. The drinkers were constructed from inverted glass graduated cylinders, with attached glass spouts, the spouts protruding into the cages, and enclosed within individual horizontal metal cylinders of approximate diameters of 2 inches, to prevent the rats from walking on the spout openings and thus causing loss of fluid from the openings on the upper faces of the spouts.

Cylindrical metal feeders 3 inches x 3 inches were used, which had removable telescoping tops with a 1.5 inch hole. The outside bottoms were leaded to prevent overturning of the feeders. Food was not spilled because of the restricted entrance.

The rats were separately offered in these feeders: yeast (Fleischmann's dried

brewer's yeast, Type 2019), dextrose (Corn Products "Ceralose"), casein (Labco vitamin-free, 100 mesh), and salt mixture (SMACO, USP XI). Roughage was offered as filter paper scraps, in a wire mesh feeder attached to the cage. The rats were offered the following fluids, from inverted 250 ml. or 100 ml. graduated cylinders, with spout drinkers: 3 per cent NaCl (USP) 250 ml., distilled water 250 ml., and a mixture of corn oil (Corn Products "Mazola") and codliver oil (USP) in the proportion of 4 to 1, 100 ml.

Preliminary experiments with some twenty rats were performed, in which various salt solutions were offered in different 100 ml. drinkers, including: water, 3 per cent NaCl, 1 per cent KCl, 2.5 per cent $MgCl_2$, 4 per cent NaH_2PO_4, 2.4 per cent Ca-lactate (or pyruvate or levulinate), corn oil, and codliver oil. These experiments were designed to reproduce some of the conditions imposed by Richter's earlier experiments on salt appetite after adrenalectomy (8). The solid salt mixture was not offered in these experiments. The rats selected successfully in a few cases, as judged by comparison of their growth curves with those of comparable rats fed Purina fox chow or an adequate mixed diet, but subsequent experiments showed that more consistent and reproducible results were obtained when the solid salt mixture was offered in place of the multiple choice of solutions. We therefore later offered only water and 3 per cent NaCl solutions, since essential minerals were available in the complete salt mixture and in the yeast.

Prior to self-selection experiments, the animals were fed on our stock diet; Purina fox chow supplemented once weekly with greens.

The food and fluid intakes, and the body weights were measured at the same time of day every other day for long periods of time, in order to establish the time at which relatively constant and comparable food and fluid intakes were established. After this time, one group of animals was bilaterally adrenalectomized, and the other was either sham-operated or unilaterally adrenalectomized.

The procedure of adrenalectomy has been described elsewhere (11), with certain modifications. Employing ether anesthesia and using the dorsal lumber approach without sterile precautions, this procedure is very similar to that recently described by Richter (7). Such bilaterally adrenalectomized rats, of the particular strain used, survived on the average of four to seven days for 100 gram rats, and thirteen to thirty days for mature rats, when they were fed a low sodium diet made of vitamin-free casein, dextrose, yeast, codliver oil, corn oil, and a sodium-free modification of the salt mixture described by Hubbell et al. (12), in which potassium salts were substituted for the sodium salts described by these authors. The animals used in the experiments to be described generally all succumbed at the conclusion of the experiment, when sodium chloride or adrenal cortical hormone therapy was withheld. The rare exceptions which did not succumb at this time were carefully autopsied and examined for accessory adrenal cortical tissue or rests, in order to see if the experiment was thereby invalidated.

In some experiments which will be described, diets were mixed in proportions dictated by changes in intake seen in self-selection experiments with adrenalec-

tomized rats, and fed to a uniformly selected group of eight to ten rats, as compared with another group fed a diet composed of normal ratios of dietary constituents, such as: dextrose 58 per cent, casein 21, corn oil 7, codliver oil 2, yeast 10, salt mixture 2, and containing 4.2 calories per gram. These experiments were designed to see if diets made according to the diets selected by adrenalectomized rats, would benefit adrenalectomized rats or alter their voluntary intake of sodium chloride.

In all, six successful self-selection experiments were performed, involving some eighty rats, over a two-year period. Each experiment involved two groups of rats, one control and one experimental group. The rats of each group were housed together in one cage, and consisted of from three to ten rats each, depending upon the type of experiment. The experiments lasted an average of three to four months each. In this way food and fluid intakes represented the average of all the rats of one group rather than individual rats.

In general, adrenalectomy was not performed until the rats had established constant food and fluid intake levels. This period varied from twenty to thirty days in most cases. Intakes and body weights were nevertheless recorded for this period every two days. Richter (7) usually found constant levels established by the fortieth day.

Of the experiments performed, only one will be described in detail, since the experiments were all essentially similar, and revealed certain consistencies exemplified by this one.

EXPERIMENTAL. *Experiment 4.* Three month old female rats averaging 156 grams body weight (range 140 to 170) were divided into two groups of ten rats each, and each group was separately caged, and offered casein, dextrose, yeast, salt mixture (dry), water, corn-codliver oil mixture, and three per cent NaCl, as described in METHODS.

The body weights and food fluid intakes were recorded every two days for a period of ninety days. The solutions in the drinkers, and the food in the various feeders were changed for fresh supplies every two days. The amount of evaporation from the spouts of the drinkers in the two day period, was neglected.

The rats of group 1, the controls, were unilaterally adrenalectomized, and those of group 2, the experimentals, were bilaterally adrenalectomized on the forty-seventh day. The operation was performed at this late time because prior to this time the water intake was not equal in the two groups, as seen in figure 1. Twenty days after the operation, a fifty-milligram pellet of desoxycorticosterone acetate[2] was subcutaneously implanted in the shoulder region of the rats of group 2, and removed twenty-five days later, and the rats subsequently given the low sodium mixed diet previously described, in addition to 0.1 per cent KCl drinking fluid, which was increased to 0.25 per cent a week later. The food intakes were averaged as per cent calories per rat per day, and the water and NaCl solutions were recorded as milliliters per rat per day. The caloric equivalents per gram of the diets selected by the rats were assumed to be: dextrose 3.75, casein 4.40, oils 9.30 (13) and yeast 2.0 (assumed 50 per cent protein, and

[2] Supplied by Roche-Organon, Inc., through the courtesy of Dr. R. D. Shaner.

negligible in fats and carbohydrate, according to analyses obtained from Fleischmann's).

The results were averaged for five-day intervals for convenience in graphing, and the results are presented in figure 1.
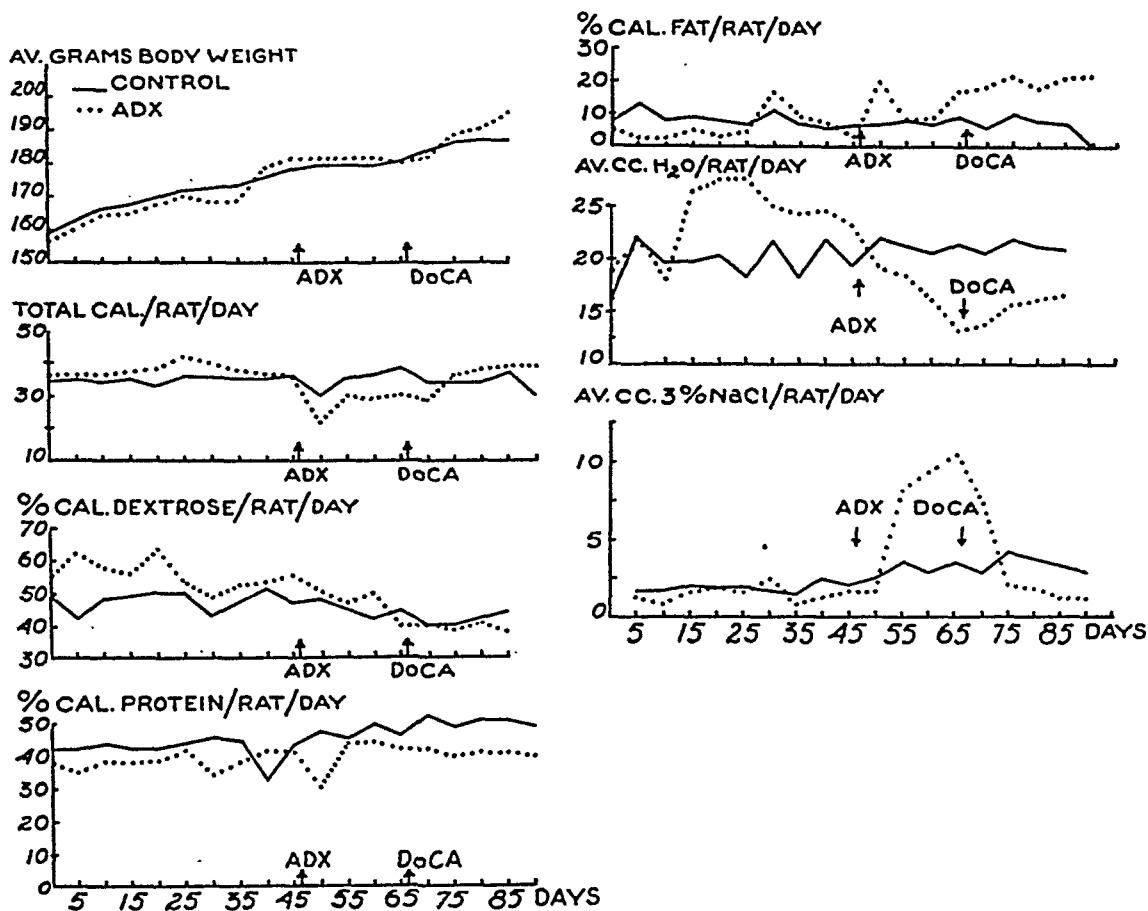
Fig. 1. Effect of adrenalectomy on voluntary selection of dietary constituents separately offered.

Constituents separately offered were dry dextrose, yeast, casein, and salt mixture; corn oil-codliver oil (4:1), 3 per cent NaCl, and water. Ten rats per group. Each group caged together. Each graph average of the 10 rats.

ADX—adrenalectomized. DoCA—Desoxycorticosterone acetate.

As can be seen from figure 1, the body weight curves showed no effect of the operations. After removal of the desoxycorticosterone acetate pellets, the animals died in 100 per cent of the cases when they had no access to salt, after an average of twenty-six days (range 9 to 36). The average body weights are not plotted after pellet removal since the scattered times invalidated the significance of such averages.

The total caloric intake showed a small transitory decrease caused by the bilateral adrenalectomy in group 2, which slowly returned to normal, especially after desoxycorticosterone acetate administration. Otherwise no significant difference was seen between the two groups.

The percent calories of dextrose showed no appreciable difference between the two groups after adrenalectomy, and the same lack of effect was seen in the protein (including yeast) and fat intakes, although transitory variations in fat intake occurred. Since the caloric equivalent of fat is high, the actual intake in milliliters was exaggerated by multiplication by this factor, 9.3, and the variations were much less noticeable if milliliters per rat per day were recorded instead of calories per cent per day. There was no significant change in the intake of the dry salt mixture, which was consumed on the average of about 100 mgm. per rat per day, but which showed considerable variation from day to day.

The water intake curves were not comparable between the two groups prior to a few days before the operations. After operation, the water intake of the bilaterally adrenalectomized rats decreased from 20 to approximately 13 ml. per rat per day, while the 3 per cent NaCl intake increased from 2 ml. per rat per day, to a maximum of 10 ml. per rat per day, a fivefold increase due to adrenalectomy, in confirmation of Richter. After desoxycorticosterone acetate administration the salt intake again returned to the pre-adrenalectomy level, while the water tended again to increase. Due to the difference in water intakes of the two groups prior to adrenalectomy, it might be believed that the changes in water intake after operation have no significance in this experiment. This may be true, but since the same tendency to decrease water intake and increase 3 per cent NaCl after adrenalectomy, was seen in the other experiments, we believe that the water decrease tends to parallel the salt increase.

The results show that we could confirm Richter's observations that adrenalectomy increases voluntary intake of sodium chloride, but we could not confirm his observation that selection of sugar decreases.

The results of the other experiments were similar to this one, with some exceptions. These exceptions were that upon occasion, the two groups of rats would sometimes not establish comparable intake levels of fluids and foods prior to adrenalectomy, as in the case of water in experiment 4, described above. In all, however, the experiments showed essentially the same results, namely, that adrenalectomy increases the voluntary intake of sodium chloride, but does not significantly alter the voluntary intake of any of the other dietary constituents, with the possible exception of water.

An outstanding exception was experiment 2, in which there were seven rats per group and the methods used were essentially the same as those described in experiment 4. In this experiment, the total caloric intake remained about the same after adrenalectomy and after desoxycorticosterone acetate or adrenal cortical extract administration. As in experiment 4, the NaCl intake increased after adrenalectomy, and decreased after desoxycorticosterone acetate, but the water intake remained the same. The fat intake, however, markedly increased and the dextrose intake markedly decreased after adrenalectomy in such a way as to render the total caloric intake unchanged. The decreased dextrose intake is in agreement with Richter's experiments, but in disagreement with our other experiments. In this particular experiment, however, after adrenalectomy, even though the rats had access to and voluntarily increased their NaCl intake,

it was noticed that their body weights fell lower than the controls, and several rats died during the time with severe diarrhea. All the rats of this group were markedly asthenic. This type of result has been described by Mark (14), whose work is described in the discussion below. In no other experiment, in which all the rats were healthy and vigorous, could a change in food intake be observed for any of the dietary constituents except NaCl (and possibly water).

On the occasions in which experiments showed changes in voluntary intake of the dietary constituents due to unknown causes or to poor condition as in experiment 2, it was thought of possible interest to mix synthetic diets, with their proportions of constituents dictated by these self-selection experiments. The first such experiment consisted of six groups of five female rats per group, average body weight 160 grams. Three groups, the controls, were fed a diet dictated by normal rats in a previous self-selection experiment, and three groups, the experimentals, on a diet dictated by adrenalectomized rats in the same experiment, in which the rats selected a high protein, low carbohydrate diet. In this experiment, as in experiment 2, the rats did poorly, and did not reversibly alter their voluntary NaCl intake after adrenalectomy and subsequent hormone therapy. The three control groups were fed a mixed diet consisting of: dextrose 58 grams per cent, casein 21, corn oil 7, codliver oil 2, yeast 10, and salt mixture 2, and contained 4.16 calories per gram, and the calories per cent of the various constituents were: carbohydrate 52, protein 28, and fats 20. The experimental groups were fed a diet consisting of: dextrose 28 grams per cent, casein 40, corn oil 10, yeast 10, codliver oil 2, salt mixture 2, and celluflour 8; containing 4.15 calories per gram, and consisting of: carbohydrate 25 calories per cent, protein 48, and fats 27.

The three control groups and the three experimental groups were given, respectively, tap water, 0.5 per cent NaCl, and 1.0 per cent NaCl as drinking fluid, and the body weights, food and fluid intakes were recorded for a week, after which the experimental groups were adrenalectomized, and the data recorded until death. Survival times were noted.

No differences were observed for food and fluid intakes, or for body weight changes after adrenalectomy, except that the rats on 0.5 and 1.0 per cent NaCl as drinking fluid ate and gained more weight than those on tap water, which is to be expected. After removal of the NaCl, no differences were noted in survival times between the control and experimental groups. This experiment indicates that the high protein, low carbohydrate diet dictated by the previously conducted self-selection experiment mentioned, had no beneficial effects on the rats with respect to appetite, weight maintenance, or intake ("requirement"?) of NaCl, as compared with a control diet.

In another similar experiment, four groups of eight female rats each, averaging 125 grams (range 117–138) were selected. The first group received a mixed diet dictated from the results of self-selection experiment 2, described above, in which dextrose intake decreased, and fat intake increased after adrenalectomy (but in which this change seemed to parallel a poor condition of the animals). The control groups received a mixed diet, diet 1, consisting of dextrose 62 grams

per cent, casein 20, yeast 12, corn oil 3.2, salt mixture 2.8, and containing 3.7 calories per gram, with the calories per cent of carbohydrate 62, protein 30, and fat 8; while the experimental group received a high fat, low carbohydrate diet, diet 2, consisting of dextrose 25 grams per cent, casein 20, corn oil 17.2, yeast 12, salt mixture 2.8, celluflour 23, and containing 3.7 calories per gram, with the calories per cent of carbohydrate 31, protein 26, and fat 43. Each rat received 2 drops of Oleum percomorphum (Abbott) once weekly as a source of vitamins A and D.

Both groups, in addition to receiving the diets described, were allowed a voluntary selection of two drinking fluids, tap water and 3 per cent NaCl, from two drinkers. For roughage, both groups had access to filter paper scraps. It was noted, however, that the rats of the experimental group did not consume the paper while those of the control group did. This was presumably because of the lack of roughage in the control diet (self-selection?). One control and one experimental group were then adrenalectomized, leaving the other two as non-adrenalectomized controls. The adrenalectomized animals were operated three weeks prior to the experiment, and maintained on salt therapy consisting of a 1 per cent NaCl, 0.2 per cent NaHCO₃ drinking fluid. Just prior to the presentation of the experimental diets, these animals were tested for completeness of adrenalectomy by withholding salt therapy, and all the rats showed a marked weight loss. After this, they were given 2 mgm. desoxycorticosterone acetate in oil subcutaneously, which restored them to normal weight and health. Thus the rats were proven, prior to the experiment, to be capable of developing a severe uniform adrenal insufficiency.

Body weights, food and fluid intake, and voluntary intake of salt or water were recorded daily. After 32 days, the 3 per cent NaCl solution was removed, and the weight loss and survival noted.

The "dictated" diets had no effect on food intake, water intake, or salt appetite for the 32 day experiment, nor were there any differences in survival after withdrawing salt, the survival time being 6 to 7 days, respectively, for the two groups of adrenalectomized rats on the two diets. It is noted that the adrenalectomized rats did select much more NaCl than the controls, in confirmation of the self-selection experiments, and those of Richter. The controls selected an average of 200 mgm. NaCl per rat per day, while the adrenalectomized rats selected an average of 400 mgm. per rat per day.

Another similar experiment was performed with mature female rats, in contrast to the young rats used in the experiment just described, with essentially the same results.

DISCUSSION. In the self-selection experiments described above, all of the dietary constituents were separately offered in different feeders and drinkers, and the salts were offered as dry salt mixture, with exception of a NaCl solution. These experiments differ from those of Richter, in which voluntary dextrose and salt intakes were observed to be altered by adrenal insufficiency. In one series of experiments, Richter (7) fed a saltless stock diet in one container, and 3 per cent NaCl, 40 per cent dextrose, and distilled water from three drink-

ers, and found that adrenalectomy decreased the intake of dextrose solution, increased that of the salt solution, and decreased the water intake. Desoxycorticosterone acetate and adrenal cortical extract tended to reverse this change. In another series of experiments (6), he fed the same diet from one container, 3 per cent NaCl and water from two drinkers, and dry dextrose from another feeder, with essentially the same results.

In the experiments we performed, in which diets were made up according to the proportions of various dietary constituents selected in certain self-selection experiments in which changes in intake were observed after adrenalectomy (it is pointed out that these experiments were exceptions), no effects of altered fat, carbohydrate, or protein content of the diets were observed on appetite, body weight, salt selection, or survival of adrenalectomized rats fed such diets. This is in agreement with the experiments of Swann (15) who examined the effects of diet on the survival of 704 immature and mature adrenalectomized rats of four different strains, and was unable to find any influence on survival, of variations of carbohydrate, fat or protein content.

In addition to the experiments we have performed, and those of Richter, Warkinton (16) has described self-selection experiments in which the rats "successfully" selected an adequate diet when presented with the separate constituents of the diet. He was interested in ascertaining whether hypo- or hyperthyroidism would alter the voluntary selection of any of the dietary constituents, but obtained negative results.

Mark (14) fed rats a saltless stock diet, and 40 per cent sugar, 3 per cent NaCl, and distilled water from three drinkers, thus essentially the same technique described by Richter (7). After adrenalectomy, the NaCl intake markedly increased, returning to normal after desoxycorticosterone acetate. No mention was made of alterations in sugar and water intakes except a statement to the effect that changes in voluntary sucrose and water intakes did not show a significant pattern. Even with access to, and increased voluntary intake of the salt solution, Mark's animals frequently died 15 to 20 days following adrenalectomy, which does not confirm Richter's 100 per cent survivals under identical conditions. Mark mentions the possibility of colony differences, but his rats' failure to maintain themselves by salt selection reminds us of our experiment 2, in which, despite an increased salt intake, several rats did poorly, and even died. It is noteworthy that experiment 2 was an exception to our general observation that adrenalectomy does not alter the voluntary intake of any of the dietary constituents when they were separately offered, in confirmation of Mark. Richter (7) mentions the possibility that the decreased sugar intake observed after adrenalectomy, when sugar is offered as the solid or as a solution in addition to a saltless stock diet, is due to impaired intestinal absorption. It is a well known fact that adrenalectomized animals cannot tolerate large amounts of hypertonic solutions such as strong sugar solutions (17), and adrenalectomized rats might therefore learn to avoid such osmotic dangers when they already have access to an adequate mixed diet in addition to an extra supply of carbohydrate in the form of solid dextrose or strong solutions of dextrose. When the

dietary constituents are separated in different feeders, however, we feel that adrenalectomy does not alter the voluntary intake ("need") of carbohydrate, or any other dietary constituent except salt and water. Richter's observation of a decreased sugar intake might be explained on the basis of an impaired sodium balance (18).

SUMMARY

1. Self-selection experiments were performed on rats, in which the effects of adrenalectomy were studied, in an attempt to see if the voluntary intake of any of the dietary constituents was altered. Dextrose, salt mixture, yeast, and casein were offered in four feeders, and 3 per cent NaCl, water, and a mixture of corn oil and codliver oil from three drinkers. Several rats were caged together. The daily intakes were recorded until constant, after which adrenalectomy was performed, and the subsequent intakes recorded for a period of time, followed by adrenal cortical hormone therapy.

2. Richter's observation of an increased voluntary selection and beneficial effect of NaCl, reversed by adrenal cortical hormone therapy, was confirmed.

3. In those experiments which were considered valid, no effect of adrenalectomy was observed on the intake of protein, carbohydrate, fat, yeast, or solid salt mixture. This differs from Richter's observation of a decreased sugar intake. It is pointed out, however, that Richter's method differed in that he presented sugar in addition to a mixed diet.

4. An exceptional experiment is described which does show a decreased sugar intake after adrenalectomy, in confirmation of Richter, but it is pointed out that the rats lost weight, and several died.

5. Diets were made up in accordance with the results of self-selection experiments, such as the exception described in paragraph 4, which were considered to show changes in self-selection of dietary constituents presumably due to the poor condition of the animals after adrenalectomy. These diets varied in carbohydrate, fat and protein content, and were fed to adrenalectomized rats. Three per cent NaCl and water were also offered. No effects of these diets, "dictated" from self-selection experiments, were observed on appetite, body weight, voluntary intake of salt solution, or survival after withholding the salt solution, even though adrenalectomy did lead to an increased salt intake.

REFERENCES

(1) RICHTER, C. P. Psychosomantic Med. 3: 105, 1941.
(2) RICHTER, C. P. Ann. Rev. Physiol. 4: 561, 1942.
(3) RICHTER, C. P. Am. J. Psychiatry 97: 878, 1941.
(4) RICHTER, C. P. Endocrinology 28: 193, 1941.
(5) RICHTER, C. P. Ibid. 24: 367, 1939.
(6) RICHTER, C. P. Proc. Soc. Exper. Biol. and Med. 48: 577, 1941.
(7) RICHTER, C. P. Endocrinology 29: 115, 1941.
(8) RICHTER, C. P. AND J. F. ECKERT. Ibid. 22: 214, 1938.
(9) RICHTER, C. P. This Journal 115: 155, 1936.
(10) MacKAY, E. M. AND L. L. MacKAY. Ibid. 83: 179, 1927.
(11) MacKAY, E. M. AND L. L. MacKAY. J. Exper. Med. 43: 395, 1926.

(12) HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN. J. Nutrition 14: 273, 1937.

(13) BODANSKY, M. Introduction to physiological chemistry. John Wiley, New York. 4th ed., 1938.

(14) MARK, J. Endocrinology 31: 582, 1942.

(15) SWANN, H. G. This Journal 118: 798, 1937.

(16) WARKINTON, J. Fed. Proc. 1: Part 2: 89, 1942.

(17) REICHSTEIN, T., F. VERZAR AND L. LASZT. Nature 139: 331, 1937.

(18) CLARK, W. G. AND E. M. MACKAY. This Journal 137: 104, 1942.

THE INFLUENCE OF SERUM CHLORIDE CONCENTRATION ON THE OXYGEN CONSUMPTION OF DOGS

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In the course of work involving observations on the oxygen consumption of dogs before and after the intravenous administration of various metabolic substrates, it was found that similar injections of NaCl solutions also increased the oxygen uptake. Since physiological saline is ordinarily considered to be metabolically inert and is commonly used as a vehicle for the injection of other materials, it became of practical as well as of theoretical importance to investigate its stimulating effect upon oxygen utilization.

Previous recognition of the above influence of NaCl has been largely associated with the disturbance encountered in adrenal insufficiency. It is well known that untreated adrenalectomized animals have an abnormally low oxygen consumption but that when such animals are maintained on an adequate intake of NaCl, their rate of oxygen consumption approaches that of normal animals (1) (2) (3) (4). Similarly, it has been demonstrated (in vitro) that the Q_{O_2} of isolated tissue from rats in adrenal insufficiency is lower than that of tissue removed from adrenalectomized rats treated with NaCl (5) (6). The previous literature contains only a few scattered references to the effect of NaCl on oxygen consumption in the normal organism. Castex and Schteingart (7) reported that subcutaneous injections of NaCl in human subjects raised the basal metabolic rate from 4 to 19 per cent. Control observations using distilled water gave no increase. Davis (8) (9) noted increased oxygen consumption in dogs following intravenous NaCl administration and observed that when the animals were initially dehydrated, the effect of NaCl administration was greater.

METHODS. The work, to which the present observations were incidental, necessitated the employment of several different physiological states in dogs and gave us the opportunity to test the effect of NaCl under the most diverse conditions. Both normal and completely depancreatized dogs were used, sometimes intact and sometimes after complete abdominal evisceration. All animals were deeply anesthetized with nembutal throughout the measurement of the respiratory exchange. The latter procedure was carried out with an apparatus to be described in a forthcoming report on other work. All animals were deprived of food for 3 days before the experimental day, depancreatized dogs being deprived of insulin as well as food for the same period. Depletion of NaCl or of chloride alone was effected by the methods of Darrow and Yannet (10) (11). The depletion was carried out on the experimental day or on the preceding day. Sodium chloride was restored to the animals by the intravenous injections of

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isotonic or hypertonic NaCl. When it was desired to replace only the sodium ion, sodium phosphate was administered. Chloride, dissociated from sodium, was given as lithium chloride.

All analyses were made on arterial blood. Plasma sodium was determined by the method of Weinback (12) and serum chloride by Wilson and Ball's method (13). The whole blood lactic acid was measured by the method of Barker and Summerson (14).

RESULTS. Figure 1 is a graph of a typical experiment on an intact normal dog. There is a striking parallelism between the depletion and restoration of NaCl and the fall and rise in the rate of oxygen consumption. It should be noted that the administration to a normal undepleted dog of the amount of NaCl which was required to restore the depleted dog to normal, also resulted in a significant rise in the oxygen consumption (about 20 per cent). However, in the normal
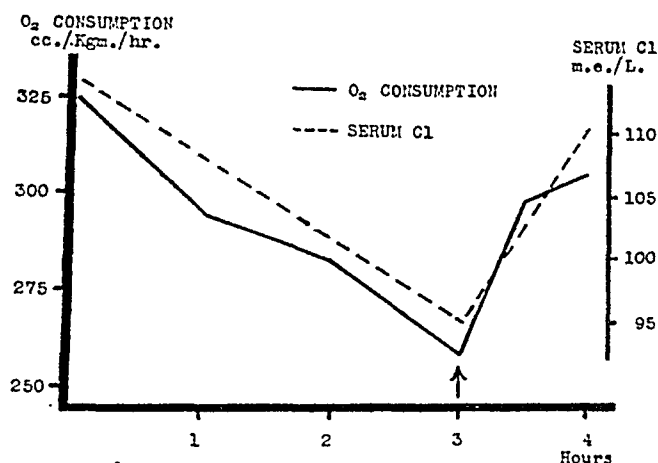
Fig. 1. A typical experiment on an intact normal dog weighing 10 kgm. It received an intraperitoneal injection of 1000 cc. of 5 per cent glucose, at zero time. The arrow represents the time of withdrawal of the fluid from the peritoneal cavity, followed by the intravenous administration of 4.7 grams of NaCl dissolved in 30 cc. of distilled water.

dog, the increase in oxygen consumption reached its peak in about 30 minutes, was maintained for another 30 minutes, and then fell to the pre-injection level about 30 minutes thereafter. In contrast to this, the administration of NaCl to a depleted dog or to an animal which was salt deficient as a result of uncontrolled pancreatic diabetes, produced an equally rapid rise in the rate of oxygen consumption, but the increased rate then persisted for as long as we continued our observations (for as long as 5 hrs.).

We have some data which indicate that the influence of NaCl on the rate of oxygen consumption is largely due to the chloride ion. By the intraperitoneal injection of a mixture of 5 per cent glucose and 150 m.e./liter $NaHCO_3$, four intact normal dogs were depleted of chloride alone. In these animals, in which the plasma sodium levels did not change, final serum chloride levels of 79, 80, 81, 83 m.e./liter were accompanied by decreases in oxygen consumption amounting to *15, 13, 43, 16* per cent respectively. Concordant results were also obtained

by depleting animals of NaCl, then administering first the sodium ion, and then the chloride ion. In three intact normal dogs, depleted to levels of *102, 117, 125* m.e./liter of plasma Na and *86, 82, 93* of serum Cl, restoration of the Na resulted in rises in oxygen consumption amounting to *10, 6, 9* per cent respectively. Subsequent restoration of the chloride increased the oxygen consumption *20, 15, 30* per cent.
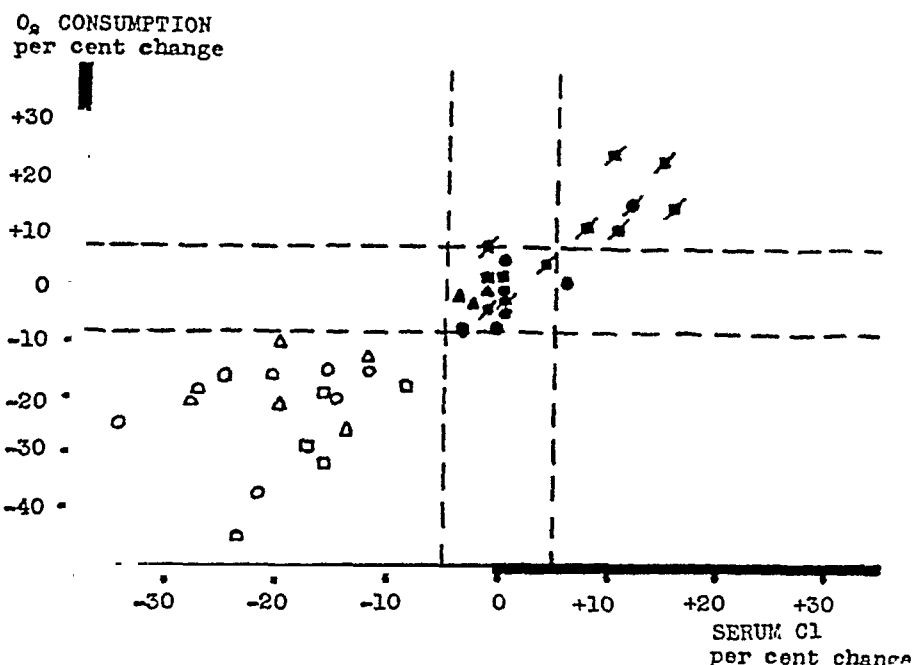
Fig. 2. A summary of all results on different animal preparations. Because of the widely different initial levels of serum chloride and oxygen consumption, the correlation shown is that between the percentage deviations from the initial levels. The deviations in chloride concentration are the result of either the abnormal state (as in the uncontrolled diabetic animals) or of the withdrawal or administration of NaCl or Cl.

Effect of NaCl depletion on normal intact dogs = O, on eviscerated normal dogs = □, on diabetic dogs = △.

Effect of Cl depletion on normal intact dogs = ◠.

NaCl restored to normal intact dogs = ●, to eviscerated normal dogs = ■, to diabetic dogs = ▲.

Additional NaCl given to normal intact dogs = ◢, to eviscerated normal dogs = ◢.

The broken lines indicate the approximate limits of normal variation.

Figure 2 summarizes all our results indicating the variation of oxygen consumption with variations in the concentration of chloride in the blood serum. Because the graph includes data from animals in a variety of physiological states, involving widely different initial levels of oxygen consumption and chloride concentration, the correlation presented is that between percentage deviations rather than absolute values. The direct relationship between serum chloride concentration and oxygen consumption in all the animals is apparent.

There was little indication from this work as to a possible explanation for the influence of serum chloride concentration on oxygen consumption. An incidental

observation which may be pertinent was the sharp rise in the levels of lactic acid in the blood, in a few sodium chloride depleted animals in which this was followed. The increases varied from 31 to 106 mgm. per cent. This suggested the possibility of the occurrence of a relative anoxia in the tissues of the depleted animals which might point to a defect in the oxygen-carrying or transfer mechanisms.

SUMMARY AND CONCLUSIONS

1. The influence on the total oxygen consumption of dogs of the administration of NaCl and of NaCl depletion was observed under a variety of conditions, including normal and depancreatized animals, both intact and after complete abdominal evisceration.

2. Under all these conditions, the oxygen consumption of the animals, if normal to begin with, was temporarily increased by the administration of NaCl. The total oxygen consumption of animals depleted of NaCl was abnormally low, and was permanently restored to the normal value by the administration of sufficient NaCl to bring the blood level back to normal.

3. Some of the data indicate that the influence of NaCl is largely due to its Cl ion.

4. There is a suggestion that the chloride ion is involved in the mechanisms for the transport and transfer of oxygen from blood to tissue.

5. From a practical standpoint, it is important to consider serum chloride concentration as a possible variable factor in all investigations involving measurement of the oxygen consumption of animals.

REFERENCES

(1) HARROP, G. A., A. WEINSTEIN, L. J. SOFFER AND J. H. TRESCHER. J. Exper. Med. **58:** 1, 1933.
(2) GROLLMAN, A. The adrenals. Williams & Wilkins Co., Baltimore, 1936.
(3) RUSSELL, J. A. This Journal **128:** 552, 1939.
(4) BROWNELL, K. A. AND F. A. HARTMAN. Endocrinology **29:** 430, 1941.
(5) RUSSELL, J. A. AND A. E. WILHELMI. J. Biol. Chem. **137:** 713, 1941.
(6) TIPTON, S. R. This Journal **132:** 74, 1941.
(7) CASTEX, M. R. AND M. SCHTEINGART. Compt. rend. Soc. de Biol. **111:** 400, 1932.
(8) DAVIS, H. A. Science **81:** 493, 1935.
(9) DAVIS, H. A. Proc. Soc. Exper. Biol. and Med. **33:** 242, 1935.
(10) DARROW, D. C. AND H. YANNET. J. Clin. Investigation **14:** 266, 1935.
(11) YANNET, H. J. Biol. Chem. **136:** 265, 1940.
(12) WEINBACK, A. P. J. Biol. Chem. **110:** 95, 1935.
(13) WILSON, D. W. AND E. B. BALL. J. Biol. Chem. **79:** 221, 1938.
(14) BARKER, S. B. AND W. H. SUMMERSON. J. Biol. Chem. **138:** 535, 1941.

THE INFLUENCE OF SODIUM CHLORIDE CONCENTRATION ON THE IN VITRO OXYGEN CONSUMPTION OF RAT DIAPHRAGM, IN THE PRESENCE AND ABSENCE OF RED BLOOD CELLS

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Previous authors have reported inconsistent and contradictory effects on the in vitro oxygen consumption of tissues as a result of varying the NaCl concentration of the medium (1) (2) (3) (4). Our own previous observations on the in vivo oxygen consumption of dogs have shown a consistently direct relationship between the concentration of the serum chloride and the basal oxygen consumption (5). It therefore seemed worthwhile to reinvestigate the subject under in vitro conditions, in an attempt to discover the reasons for the lack of correspondence of such results with those we obtained in the living organism.

METHODS. The oxygen consumption of portions of the diaphragms of 60 to 90 gram rats was measured by the Warburg technique. A calcium-free Ringer-phosphate buffer, pH 7.4, containing 200 mgm. per cent glucose was used as the medium. The basic solution to which glucose and NaCl were added as required, contained the following concentrations of ions, expressed as m.e./liter: K = 4.4, Mg = 1.2, Na = 30, Cl = 3.0, SO_4 = 1.2, PO_4 = 20. Both glucose and NaCl were added in dry, solid state to aliquots of the basic solution to yield the following 3 final solutions: Solution no. 1 in its final form contained 150 m.e./liter of Na and 133 m.e./liter of Cl; solution no. 2 contained 200 m.e./liter of Na and 183 m.e./liter of Cl; solution no. 3 contained 100 m.e./liter of Na, 83 of Cl, and (to avoid hypotonicity) 75 m.e./liter of sucrose. For convenience, these solutions are hereinafter designated as NaCl at 150, 200 and 100 m.e./liter respectively.

Every experiment was performed in duplicate for an observation period of one hour and consisted of a comparison of control vessels containing the physiologically normal NaCl concentration of 150 m.e./liter (solution no. 1) with test vessels containing solutions no. 2 and 3. In each experiment, portions from the same diaphragms were compared in the different solutions. Q_{O_2}'s were determined on the basis of dry weight of the tissues.

Two other variables tested in these experiments were the gas phase (100 per cent oxygen compared with air) and the presence or absence of red blood cells in the medium. When red blood cells were used, they were obtained from oxalated, defibrinated, heparinized or citrated dog or human blood. They were washed 5 or 6 times with 0.9 per cent NaCl solution, oxygenated in a separate vessel, and added to the medium in the ratio of 1 cc. of packed cells to 5 cc. of

the medium. A similar proportion of red blood cells was added to the thermo-barometers in these experiments.

RESULTS. (a) *Lack of influence of NaCl concentration on the Q_{O_2} of rat diaphragm, when the gas phase was 100 per cent oxygen, and in the absence of red blood cells.* Table 1 clearly shows that under the conditions commonly used for in vitro work, the concentration of sodium chloride in the medium (within reasonable limits) has little or no effect on the Q_{O_2}. It therefore seemed possible that the definite effects of NaCl seen in our previous in vivo work resulted from an exchange of ions between the blood serum and the tissue cells, which for some reason or other, did not occur under our in vitro conditions.

(b) *The influence of in vivo NaCl depletion on the Q_{O_2} of rat diaphragm, gas phase = 100 per cent oxygen, red blood cells absent.* In view of the above results

TABLE 1

Comparison of the Q_{O_2} of portions of the same rat diaphragms, showing the lack of influence of varying NaCl concentrations when the gas phase is 100 per cent oxygen and no red blood cells are present

CONDITIONS	Q_{O_2}		
	NaCl 100 m.e./liter	NaCl 150 m.e./liter	NaCl 200 m.e./liter
100 per cent oxygen without red blood cells	6.84	6.66	
	6.46	6.50	
	6.42	6.36	
	6.29	6.19	
		6.05	6.10
		6.30	6.25
		6.40	6.32
		6.36	6.50
Average Q_{O_2}............	6.50	6.35	6.39
Per cent difference......	+2	0	+1

it became of interest to determine the Q_{O_2} of diaphragmatic muscle of rats which had been depleted of NaCl during life. Others had previously shown that the tissue of rats in adrenal insufficiency had a lowered oxygen uptake in vitro, while the tissues from adrenalectomized rats adequately treated with NaCl had normal Q_{O_2}'s (6) (7). It had also been shown that animals may be depleted of NaCl by injecting isotonic glucose intraperitoneally, and these animals suffer the same electrolyte and water changes in blood and muscle as occur in adrenal insufficiency (8) (9). We employed the latter technique to obtain NaCl depleted rats. By trial and error it was found that the necessary depletion, in 60 to 90 gram rats, resulted from the intraperitoneal injection of about 20 cc. of 5 per cent glucose in water. The accumulated fluid was withdrawn from the peritoneal cavity at the end of four hours. The animals were placed in a cage with free access to water but no food, and sacrificed for the experiment 8 hours thereafter.

TABLE 2

The influence of NaCl concentration on the Q_{O_2} of rat diaphragm in the presence of blood cells, gas phase = air, and the lack of influence of NaCl when oxygen is substituted for air

Portions of the same rat diaphragm are compared in each case

	Q_{O_2}		
CONDITIONS	NaCl 100 m.e./ liter	NaCl 150 m.e./ liter	NaCl 200 m.e./ liter
Air and red blood cells	4.20	6.65	
	4.19	5.19	
	4.57	5.95	
	4.32	5.75	
	4.34	6.06	
	5.90	6.48	
	4.09	5.99	
	3.92	6.01	
	4.63	5.92	
	4.74	5.87	
		6.28	7.54
		5.97	6.71
		5.74	6.81
		5.77	6.70
		5.86	6.67
Average..........	4.49	5.96	6.89
Per cent difference....	−25	0	+16
100 per cent oxygen and red blood cells	7.94	7.44	
	6.92	6.85	
	8.10	8.76	
	7.80	7.87	
		7.09	7.54
		6.16	6.48
		6.01	6.15
		7.85	8.36
		8.43	8.23
Average..........	7.69	7.49	7.35
Per cent difference....	+3	0	−2

TABLE 3

The lack of influence of osmotic pressure on the Q_{O_2} of rat diaphragm in the presence of red blood cells, gas phase = air or oxygen

Portions of the same rat diaphragm are compared in each case

	Q_{O_2}		
CONDITIONS	NaCl— 150 m.e./ liter Sucrose— 75 m.e./ liter	NaCl 150 m.e./ liter	NaCl— 150 m.e./ liter Glucose— 75 m.e./ liter
Air and red blood cells	5.39	5.55	
	5.90	5.99	
	5.66	5.59	
	5.98	5.88	
	5.79	6.06	
		5.99	6.19
		5.69	5.58
		6.20	6.68
		5.89	5.90
Average..........	5.81	5.77	5.87
Per cent difference....	+1	0	+1
100 per cent oxygen and red blood cells	8.10	7.30	
	7.32	7.36	
	7.80	7.19	
	6.78	6.79	
		7.59	7.79
		5.58	5.69
		8.20	8.78
		7.19	6.90
Average..........	7.16	7.15	7.29
Per cent difference....	0	0	+2

The Q_{O_2}'s of the diaphragms of these rats were determined at the physiologically normal concentration of 150 m.e./liter of NaCl.

The oxygen consumptions of the diaphragms of three such animals, done in

duplicate, were as follows: 4.51, 5.08, Av. = 4.79; 5.69, 5.59, Av. = 5.64; 5.36, 5.35, Av. = 5.36. These values are significantly lower than the Q_{O_2}'s of normal rats at the same NaCl concentration, as shown in table 1.

These results seemed to confirm the difference between the living animal and tissue in vitro as regards the effect of varying the NaCl concentration, but offered no explanation. It occurred to us that another difference between the two sets of conditions was the presence of red blood cells in the living system and their absence in vitro.

(c) *The influence of NaCl concentration on the Q_{O_2} of rat diaphragm in the presence of red blood cells, gas phase = air.* The data in table 2 show that when in vivo conditions are more closely approximated (in the presence of red blood cells, gas phase = air) the influence of NaCl concentration on the Q_{O_2} of isolated muscle can be demonstrated. The effect of the NaCl is apparently exerted on the tranfer of oxygen from the red blood cells to the acceptors in the muscle, for when 100 per cent oxygen is substituted for air, the NaCl concentration loses its effect. This explains why the influence of NaCl was so readily demonstrable in the living organism, but did not manifest itself under the ordinary conditions of in vitro work. The amount of oxygen dissolved in the medium, when it is in contact with 100 per cent oxygen, must be at or above the maximum which obtains in the blood plasma of the living organism.

The mechanism by which NaCl influences the transfer of oxygen from red blood cells to tissue is somewhat clarified by other of our findings. The altered osmotic pressure of the medium does not appear to be a factor since equi-osmotic amounts of glucose or sucrose were without significant influence (table 3). However, it was found that a solution of hemoglobin, obtained by laking red blood cells and discarding the cellular elements, could substitute for red blood cells in demonstrating the influence of NaCl. For example, the differences in Q_{O_2} between NaCl concentrations of 100 and 150 m.e./liter in the presence of hemoglobin, gas phase = air, were as follows: 4.09 vs. 5.66, 4.51 vs. 5.68, 4.55 vs. 5.89, 4.90 vs. 6.06, Av. = 4.51 vs. 5.82. It seems likely, therefore, that NaCl acts by influencing the rate of dissociation of oxygen from hemoglobin and hence its availability to the tissues. The influence of NaCl on the dissociation of oxyhemoglobin has been previously demonstrated (10).

SUMMARY

The influence of the concentration of NaCl in the blood plasma upon oxygen consumption previously demonstrated in living animals, has been paralleled in vitro. The in vitro demonstration is possible only when the conditions more closely approximate the physiological than they ordinarily do. The effect is not apparent when tests are made in the absence of red blood cells or hemoglobin, and when the gas phase is 100 per cent oxygen. It is readily demonstrable in the presence of red blood cells or hemoglobin, and when the gas phase is air. Substituting oxygen for air in the latter case abolishes the effect. It is concluded that the NaCl acts both in vivo and in vitro (under physiological conditions),

by influencing the rate of dissociation of oxygen and hemoglobin, and thus affecting the amount of oxygen available for use by the tissues.

REFERENCES

(1) CHANG, T. H., M. SHAFFER AND R. W. GERARD. This Journal 111: 681, 1935.

(2) DICKENS, F. AND G. D. GREVILLE. Biochem. J. 29: 1468, 1935.

(3) ELLIOTT, K. A. C. AND B. LIBET. J. Biol. Chem. 143: 227, 1942.

(4) CANZANELLI, A., G. ROGERS AND D. RAPPORT. This Journal 135: 309, 1942.

(5) COHN, C. AND S. SOSKIN. This Journal 139: 80, 1943.

(6) TIPTON, S. R. This Journal 132: 74, 1941.

(7) RUSSELL, J. A. AND A. E. WILHELMI. J. Biol. Chem. 137: 713, 1941.

(8) DARROW, D. C. AND H. YANNET. J. Clin. Investigation 14: 266, 1935.

(9) MUNTWYLER, E., R. C. MELLORS AND F. R. MAUTZ. J. Biol. Chem. 134: 345, 367, 1940.

(10) SIDWELL, A. E., JR., R. H. MUNCH, E. S. G. BARRON AND T. R. HOGNESS. J. Biol. Chem. 123: 335, 1938.

SOME FACTORS AFFECTING AUGMENTATION OF PITUITARY GONADOTROPIC EXTRACTS BY HEME[1]

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The effect of the gonadotropic complex of the pituitary gland of certain animals is increased when it is injected with various substances which are themselves inactive in the stimulation of the ovaries of immature rats. These substances or augmenters include tannic acid (1), copper (2) and zinc salts (3, 4, 5), yeast extract and ash from yeast (2), male urine (6), blood serum (7), leucocytes (8), egg albumin (9), merthiolate (10) and chlorophyll (11, 12). Casida (13) found that the formed elements of blood augment gonadotropic extracts of sheep pituitary powder while McShan and Meyer (14, 15) separated the hemoglobin from the blood of the cow into heme and globin and found that the heme was an effective augmenter of the gonadotropic hormone of sheep, hog, man, cow and chicken pituitary glands.

These augmenters were usually tested by administering them with a basic dose of the gonadotropic extract which would when given alone stimulate the ovaries of immature rats measurably. As a rule the assays of pituitary gonadotropic extracts have been based on the administration of varying amounts over a constant period of time, and in testing for augmentation a known amount of the extract was combined and given with a known amount of the augmenter over the same period of time. However, in regard to the assay of gonadotropic extracts Fluhmann (16) gave a constant amount of sheep pituitary extract to immature rats over 5, 10, 15 and 20 days, and found that the ovarian weights were greater at 5 days than at 10, 15 and 20 days. Deanesly (17) found that when the injection period and amount of pituitary gonadotropic extract given to immature rats were varied the ovaries increased in weight during the first 10 days, were rather constant from 10 to 20 days, but decreased in weight during the 20 to 30 day period.

No systematic study has been made therefore of the ovarian weight response produced in immature rats by the pituitary gonadotropic complex when the amount of hormone, the period of administration and the age of animals are varied, and when under the same conditions the hormone is augmented by an effective augmenter such as heme. As far as we are aware no report has been made on the relationship of the fresh weight to the dry weight of the ovaries stimulated by the extract and those stimulated by the combination of the extract and augmenter, and the effect that corpora have on this fresh weight-dry

weight relationship. This report is concerned with the presentation of the results obtained in the study of these factors and relationships.

An experiment was devised to study the effect of four different experimental factors upon the ovarian reaction of an unfractionated aqueous extract of pituitary. These factors were dosage of pituitary, presence or absence of a quantity of hemin[2] in the injection preparation, total number of injections, and age of the experimental rats. Four different dosages of pituitary extract were used: 3, 6, 12 and 24 mgm.-equivalent of acetone desiccated sheep pituitary per injection. One half of the rats received 0.5 mgm. hemin per injection mixed with the pituitary extract; the other half received no hemin. A comparison was made between rats 21 days of age and those 31 days of age at the beginning of the

TABLE 1

Average ovarian weight and percentage ovarian solids for pairs of rats on different treatments

MGM. PER INJ.		NO. OF INJS.									
		4		8		12		16		20	
		Age (days)									
Pit. extr.	Hemin	21	31	21	31	21	31	21	31	21	31
		Average ovarian weight, mgm.—Average percentage ovarian solids									
3	0.0	14–21.4	23–23.6	14–20.4	21–21.0	12–24.4	20–22.5	11–23.6	14–23.6	10–23.5	25–22.8
	0.5	22–19.0	25–21.4	36–15.6	38–19.8	38–15.8	52–21.8	82–14.9	47–21.4	34–17.6	150–20.8
6	0.0	20–20.4	25–20.7	21–20.8	54–18.0	22–21.0	74–18.6	30–22.1	40–21.0	22–21.9	58–21.7
	0.5	32–18.0	50–18.4	106–15.4	126–16.8	266–13.3	162–18.0	378–16.9	258–15.8	580–16.4	372–18.4
12	0.0	22–20.4	45–18.1	50–18.8	74–18.8	50–21.1	88–19.6	44–22.2	59–20.4	49–20.0	121–19.9
	0.5	48–17.4	56–17.0	143–15.8	190–16.4	355–15.6	346–16.4	434–16.6	589–17.0	474–18.6	698–17.8
24	0.0	34–17.4	60–17.0	78–18.2	134–17.9	84–21.2	152–20.3	64–21.5	101–20.0	68–20.8	107–19.6
	0.5	50–16.7	108–14.1	145–16.4	180–16.3	252–16.2	277–17.2	246–18.3	431–18.2	372–17.6	556–19.4

experiment. Five different numbers of injections were used, namely, 4, 8, 12, 16 and 20 (2, 4, 6, 8 and 10 days of injection).

The animals were injected twice daily, in the morning and evening, with $\frac{1}{2}$ cc. of the proper preparation and all injections were made subcutaneously. Autopsy was performed approximately 12 hours after the last injection. After the ovaries were removed and weighed, they were placed in a desiccator over sulphuric acid and left at room temperature until they came to a constant weight. From this treatment the percentage of solids was determined.

One hundred sixty rats (80 random pairs) were used in this experiment; no two pairs of rats received exactly the same treatment with respect to all four factors (table 1). In other words, all possible combinations of treatments were made. This meant that all dosages of pituitary were combined with all different

[2] The hemin was dissolved in weak sodium hydroxide, thus yielding "heme" which is the augmenting form of the substance.

numbers of injections for both age groups, with and without addition of hemin to the pituitary preparations. There was no intention of establishing the significance of the difference between any particular combination of treatments and any other particular combination. Only the more generalized effects of the factors under study were being sought.

It is not known definitely that the percentage of ovarian solids varies directly with the degree of luteinization. A determination was made of solids, however, in the belief that it gives an inverse quantitative indication of the degree of follicular stimulation, the degree of which ordinarily is estimated qualitatively. This belief is presumed from the usual lower percentage of solids in body fluids than in organ tissues.

The experiment was of such a nature that the results could be studied by analysis of variance and the data will be presented from the point of view of such an analysis.

EXPERIMENTAL RESULTS AND DISCUSSION. Casual inspection of the data (table 1) would lead one to suspect that all four of the factors being studied were a source of variation both in ovarian weight and percentage ovarian solids. Calculations of the over-all range in results from the different treatments show the average ovarian weight for the 80 rats which received pituitary extract alone was 50 mgm. The addition to the extract of 0.5 mgm. hemin per injection to 80 other rats similarly treated in other respects produced 220 mgm. ovaries. The hemin-treated rats, therefore, showed ovarian weight approximately 440 per cent of the weight of those rats treated with pituitary alone. The 40 rats on each of the 3, 6, 12 and 24 mgm. dosages of pituitary, irrespective of other treatments combined with them, showed average ovarian weights of 34, 135, 196 and 154 mgm. In the experiment as a whole, therefore, there was a tendency for ovarian weight to reach a plateau at the 12 mgm. dosage level. Ovarian weights of 40, 88, 138, 176 and 232 mgm. were shown by respective groups of 32 rats on the 4, 8, 12, 16 and 20 injections. Rather uniform increases of ovarian weight occurred with the increase in number of injections throughout. The averages for the 21-day and 31-day rats showed 120 and 150 mgm. ovaries, respectively. Statistically, the probability of the variation associated with above factors, being due to chance alone was less than 0.01 (table 2—Hemin, AP, Inj., Age).

Hemin, pituitary dosage, and number of injections all produced a marked effect upon the percentage of ovarian solids. Age, however, showed an effect which was only on the margin of significance (table 2). The percentage of solids in the ovaries from rats treated with pituitary alone was calculated to be 20.6. This is to be compared with 17.4, the average percentage of solids in the ovaries of rats receiving both pituitary and hemin and a difference of 3.2 per cent. The percentages of solids for the different pituitary dosages were 20.8, 18.6, 18.4 and 18.2, respectively, for the 3, 6, 12 and 24 mgm. levels. The three higher dosages showed from 2.2 to 2.6 per cent less solids in the ovaries than the 3 mgm. dosage. The solids showed a decline from 18.8 to 17.9 per cent between the 4 and 8 injection treatment but there was an increase to 19.0, 19.6 and 19.8, respectively,

for 12, 16 and 20 injections.   As stated above, the effect of age was questionable, the percentage solids for the 21-day old rats being 18.8 and for the 31-day, 19.2

Within the limits of the different variables studied in this experiment, the variation in terms of mean square (table 2) which was produced by hemin exceeded that produced by any other one factor.   This was true both for ovarian weight and for the percentage of ovarian solids (table 2).

TABLE 2

Analysis of variance in ovarian characters

CAUSE OF VARIATION	D/F	OVARIAN WEIGHT		PERCENTAGE OVARIAN SOLIDS	
		Mean square	F	Mean square	F
Hemin....................	1	1,145,146	647.0*	431.98	332.3*
AP.........................	3	205,722	116.2*	54.83	42.2*
Inj.........................	4	178,123	100.6*	17.82	13.7*
Age........................	1	37,393	21.1*	5.07	3.9†
Hemin x AP...............	3	97,699	55.2*	5.47	4.2*
Hemin x inj...............	4	148,281	83.8*	8.30	6.4*
Hemin x age..............	1	140	0.08‡	54.64	42.0*
AP x inj...................	12	16,880	9.5*	4.82	3.7*
AP x age..................	3	20,510	11.6*	16.74	12.9*
Inj. x age.................	4	2,503	1.4‡	0.80	0.6‡
Hemin x AP x inj..........	12	13,460	7.6*	0.90	0.7‡
Hemin x inj. x age........	4	2,614	1.5‡	5.84	4.5*
Hemin x AP x age.........	3	14,263	8.1*	6.50	5.0*
AP x inj. x age............	12	4,211	2.38†	1.35	1.0‡
Hemin x AP x inj. x age.......	12	3,971	2.24†	2.00	1.5‡
Error......................	80	1,770		1.30	
Total......................	159	25,957		6.83	

Hemin = Presence and absence of hemin.
AP   = Different dosages of pituitary extract.
Inj.   = Different numbers of injections administered.
Age   = 21 and 31 day old rats.

* = P <0.01.
† = P 0.01–0.05.
‡ = P >0.05.

The various interactions in which hemin is involved (table 2) indicate the effects of the other three factors, alone or in combination, upon hemin action, i.e., upon augmentation in milligrams of ovarian weight or upon decline in percentage ovarian solids.

The effects of number of injections and pituitary dosage upon action of hemin were about equal in causing variation both in augmentation of ovarian weight and decline in percentage of ovarian solids (table 2 and fig. 1).   Age, however, in the experiment as a whole, modified the hemin effect only with respect to ovarian solids, where, as a matter of contrast, it was more effective than either pituitary dosage or number of injections.

The effects of variation in pituitary dosage upon augmentation in ovarian weight were modified about equally by age differences and differences in the number of injections. The results of differences in the number of injections on the other hand were affected by variations in pituitary dosage but not by differences in age. Age, however, although not capable of modifying augmentation in the experiment as a whole, did affect differentially the augmentation on different pituitary dosages. This was not true so far as different numbers of injections were concerned.

As stated above, age proved to be the most effective agent in modifying the effects of hemin upon ovarian solids. The effects of age, however, were in turn modified by the numbers of injections and by the pituitary dosage. If we now look at the variation in ovarian solids due to hemin but from the standpoint of the pituitary dosage, we find that it in turn is modified by variations in age but
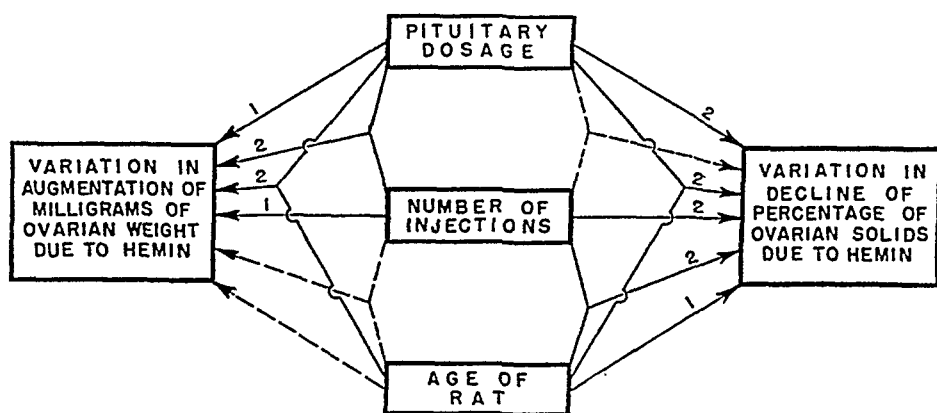
Fig. 1. Cause of variation in action of hemin upon ovarian characters. The broken arrows indicate that no significant effect of the factor was found. The branched arrows represent interaction of two factors. The numbers on the arrows indicate their rank in importance for causing variation; this ranking is based on the mean squares from table 2. A rank of 1 indicates a more important cause of variation than a rank of 2. All factors given the same rank were approximately equal in importance.

not by variations in numbers of injections. Again viewing it from the number of injections, we find that it was modified by age but not by pituitary dosage.

Augmentation in excess of 500 mgm. of ovarian weight has occurred on several different treatments within this experiment. The largest pair of ovaries obtained weighed 730 mgm. and this was in a rat 32 days of age at the time of autopsy, which had been treated for 10 days. It is believed that this degree of stimulation of the ovary is in excess of any previously reported for rats below the age of puberty.

In general, factors of an unknown sort appear to limit the attainable ovarian weight from injection of pituitary extract alone. This seems to be true whether increases in dosage are made with a constant treatment-interval or with an increasing treatment-interval. The addition of hemin to pituitary extract raises the limits for ovarian weight whether duration of treatment is held constant or is increased. The data emphasize the relatively low efficiency realized from unfractionated extracts alone and also point out that the ovary is capable of much greater reaction than is generally appreciated.

It has been shown (manuscript in preparation) that the percentage of ovarian solids is correlated with augmentation in ovarian weight (−0.70). The authors have developed the impression that there is a negative association between the percentage of ovarian solids and the degree of luteinization of the ovary. Methods of gathering objective data on the latter have not been devised, however, so the impression is a subjective one. The data support the statement, though, that hemin increases the water content of the ovary both on an absolute and a percentage basis. It is believed that this implies a modification of the physiological stimulation exerted on the ovary by the pituitary preparation. The balance between the follicle-stimulating and the luteinizing activities would appear to be altered by hemin. Greater emphasis seems to be exerted upon fluid storage in the follicles and less upon luteinization by the interacting hemin-pituitary preparation than by pituitary extract alone.

SUMMARY

The addition of hemin to pituitary gonadotropic extracts produced augmentation of ovarian weight and a decline in percentage of ovarian solids. The ability of three factors: age of test animal, dosage of pituitary extract and number of injections to cause variation in these effects of hemin was studied in an experiment involving 160 immature rats.

Variation in augmentation of ovarian weight was caused by: 1. Pituitary dosage. 2. Number of injections. 3. Pituitary dosage interacting with number of injections. 4. Pituitary dosage interacting with age of rat.

Variation in decline of percentage of ovarian solids was caused by: 1. Pituitary dosage. 2. Number of injections. 3. Age of rat. 4. Age of rat interacting with pituitary dosage. 5. Age of rat interacting with number of injections.

REFERENCES

(1) FEVOLD, H. L., F. L. HISAW, A. HELLBAUM AND R. HERTZ. This Journal 104: 710, 1933.
(2) FEVOLD, H. L., F. L. HISAW AND R. O. GREEP. This Journal 117: 68, 1936.
(3) MAXWELL, L. C. This Journal 110: 458, 1934.
(4) DEANESLY, R. J. Endocrinology 1: 307, 1939.
(5) BISCHOFF, F. Endocrinology 27: 554, 1940.
(6) HELLBAUM, A. A. Proc. Soc. Exper. Biol. and Med. 33: 568, 1936.
(7) EVANS, H. M., M. E. SIMPSON AND P. R. AUSTIN. J. Exper. Med. 58: 561, 1933.
(8) KRAATZ, C. P. This Journal 117: 250, 1936.
(9) SAUNDERS, F. L. AND H. H. COLE. Endocrinology 23: 302, 1938.
(10) CHEN, G. AND H. B. VAN DYKE. J. Pharmacol. and Exper. Therap. 62: 333, 1938.
(11) LEATHEM, J. H. AND U. WESTPHAL. Endocrinology 27: 567, 1940.
(12) BRENEMAN, W. R. Endocrinology 24: 488, 1939.
(13) CASIDA, L. E. Proc. Soc. Exper. Biol. and Med. 33: 570, 1936.
(14) McSHAN, W. H. AND R. K. MEYER. This Journal 119: 574, 1937.
(15) McSHAN, W. H. AND R. K. MEYER. Endocrinology 28: 694, 1941.
(16) FLUHMANN, C. F. Proc. Soc. Exper. Biol. and Med. 30: 1014, 1933.
(17) DEANESLY, R. Quart. J. and Year Book of Pharm. 8: 651, 1935.

RESPIRATORY CHANGES IN PULMONARY VASCULAR CAPACITY

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In motion picture studies in which respiratory changes in the output of the right and left ventricles were estimated separately in dogs with the chest closed, Shuler, Ensor, Gunning, Moss and Johnson (1942) found that during inspiration the output of the right ventricle increased, while the output of the left ventricle decreased. These inspiratory changes (which were reversed in expiration) were interpreted as due to *a*, an aspiration of blood into the right ventricle, and *b*, an increase in the pulmonary vascular capacity such that the filling and stroke volume of the left ventricle decreased.

The experiments here reported were designed to test the validity of these interpretations under somewhat simplified conditions using isolated terrapin hearts and terrapin heart-lung preparations perfused with Ringer's solution. The preparations were placed in a closed chamber representing the mammalian thorax, in which the pressure could be changed in a manner simulating the mammalian respiratory movements (see table 1).

In one series of experiments the terrapin heart alone was perfused, to simulate the right chambers of the mammalian heart, and its output measured by counting drops pumped from the aorta. When the heart was subjected to a negative pressure of one and a half inches of water (duplicating inspiration) the ventricular filling and stroke volumes increased two- to three-fold. This procedure resembled the inspiratory aspiration of blood into the mammalian right ventricle and the consequent increased output into the pulmonary artery.

In a second series the ventricle plus the lungs on its arterial side were perfused to simulate the right heart chambers plus the lungs of the mammal, and the blood flow from the pulmonary vein was measured. The flow decreased during "inspiration," showing that the increased capacity of the pulmonary vessels exceeded the increased output of the ventricle. This corresponds to a pooling of blood in the pulmonary bed in mammals during inspiration, so that the flow of blood into the left ventricle decreases even though the flow into the lungs increases.

In a third series the heart plus the lungs on its venous side were perfused to simulate the mammalian lungs and left heart chambers. In this series the output of the heart into the aorta fell when the "intrathoracic" pressure was lowered, corresponding to the lowered output of the mammalian left ventricle during inspiration. In both cases the lowered output results from decreased diastolic filling which, in turn, is a consequence of an increased fluid retention in the larger vascular bed of the lungs.

Thus, the various respiratory mechanical effects on blood flow in the mammalian chest were analyzed stepwise in three simple experiments.

In the light of these qualititative findings it seemed desirable to attempt to quantitate the respiratory changes in the capacity of the mammalian lung vessels, and to correlate them with the cyclic respiratory fluctuations in right and left ventricular output. Such observations should reveal whether or not the increased capacity of the pulmonary vessels in inspiration is sufficient to accommodate the extra blood pumped by the right ventricle in that phase of respiration.

The excised lungs of dogs were placed in a closed chamber representing the thorax. The trachea communicated with the exterior, and the pulmonary vessels, filled with blood treated with chlorozol fast pink to prevent coagulation, communicated with calibrated glass cylinders placed nearly horizontally at a height of six inches above the hilum of the lungs. The lungs were inflated by a negative "intrathoracic" pressure of four inches of water to simulate normal in-

TABLE 1

Summary of three series of experiments on terrapin hearts and heart-lung preparations, with analogies in the mammalian circulation, and the results obtained

SERIES NO.	TERRAPIN PREPARATION PLACED IN A CLOSED CHAMBER ("THORAX") AND SUBJECTED TO NEGATIVE PRESSURES ("INSPIRATION")	PREPARATION SIMULATES IN THE MAMMALIAN CIRCULATION:	FLOW IN PREPARATION MEASURED FROM:	RESEMBLES FLOW IN MAMMALIAN CIRCULATION INTO:	EFFECT PRODUCED BY LOWERING THE PRESSURE ("INSPIRATION")
1	Heart alone	Right heart	Aorta	Pulmonary artery	Increase
2	Heart, plus lungs on arterial side	Right heart and lungs	Pulmonary vein	Left heart	Decrease
3	Lungs (on venous side) plus heart	Lungs and left heart	Aorta	Aorta	Decrease

spiration, and deflated by a return to negative one inch of water pressure to simulate normal expiration. By means of changes in the meniscus of the blood in the calibrated cylinders the volume changes in the pulmonary vessels were measured.

For a ten kilogram dog these changes in pulmonary vascular capacity were 20 to 29 cc. (average 25 cc.) of blood in each inspiration or expiration. Attempts to measure these changes by other methods (Tigerstedt, 1903, 1907; Spehl and Desquin, 1909) have revealed similar volume changes.

Analysis[1] of the data published by Shuler and co-workers (1942) reveals that, in inspiration, the excess in output of the right ventricle over that of the left ventricle varies (in the respiratory cycles analyzed) between 18.7 cc. and 32.7 cc. in dogs weighing 10 kgm. and averages 27.1 cc., which closely approximates the

[1] This involves the assumptions that the average left or right ventricular stroke volume in dogs is 1.9 cc. per kgm. body weight (Henderson, 1923), and that the area changes measured by Shuler provide a means for estimating approximate volume changes.

inspiratory increase in pulmonary vascular capacity (averaging 25 cc. for a 10 kgm. dog) measured in the experiments here reported.

These observations indicate that the respiratory changes in pulmonary vascular capacity are approximately equal to the respiratory differences in the output of the right and left ventricles and lend further weight to the interpretations of Shuler and co-workers.

The changes in pulmonary vascular capacity are not apparent in the pulmonary arterioles and capillaries when these vessels are observed directly in the normally respiring mammal (Wearn, Ernstene, Bromer, Barr, German and Zschiesche, 1934). Therefore it seems likely that they occur mainly in the pulmonary veins, which are the largest pulmonary vessels, with thin distensible walls. Since Boyd and Patras (1941) have shown that even the relatively thick walled ventricles are affected by the intrathoracic pressure changes, it is likely that the pulmonary arteries also participate in the capacity changes.

Further analysis of the data of Shuler and co-workers (1942) indicates that the stroke volume of the right ventricle increased three to three and a half fold during inspiration, while the left ventricular stroke volume increased less than 50 per cent during expiration. These changes together with the changes in pulmonary vascular capacity clarify the concept of the respiratory action on the blood flow. Blood enters the lungs in increased volumes in inspiration at the time when the partial pressures of the gases in the blood differ most from those in the lungs, assuring efficient aeration. Blood leaves the lungs in a more nearly continuous flow, promoting a greater constancy of flow in the systemic vessels. If the changes in pulmonary vascular capacity occur largely in the pulmonary veins there is little if any inspiratory increase in the volume of blood in the capillaries where gaseous exchange occurs, yet there is apparently a greater volume flow through the lung capillaries during inspiration than during expiration so that efficiency of aeration is probably promoted.

SUMMARY AND CONCLUSIONS

1. Under simplified conditions, using perfused terrapin hearts and heart-lung preparations enclosed in an artificial thorax, the following effects of inspiration upon the mammalian heart were simulated:

a. Aspiration of blood into the right heart with a resulting increase in output.

b. An increased capacity of, and retention of blood in, the pulmonary vascular bed, reducing the flow of blood through the pulmonary vein.

c. A decreased filling and output of the left ventricle.

2. The changes in vascular capacity of excised dog's lungs accompanying inflation by an "intrathoracic" negative pressure of four inches of water and deflation by a return to atmospheric pressure were measured.

3. The magnitude of the increase in pulmonary vascular capacity in inspiration was found to be adequate to accommodate the extra blood pumped by the right ventricle in inspiration, as estimated from Shuler's results.

4. The respiratory changes in pulmonary vascular capacity probably occur chiefly in the veins.

REFERENCES

BOYD, T. E. AND M. C. PATRAS. This Journal 134: 74, 1941.

HENDERSON, Y. Physiol. Rev. 3: 165, 1923.

SHULER, R. H., C. ENSOR, R. E. GUNNING, W. G. MOSS AND V. JOHNSON. This Journal 137: 620, 1942.

SPEHL, P. AND E. DESQUIN. Arch. ital. de biol. 51: 23, 1909.

TIGERSTEDT, R. Skand. Arch. f. Physiol. 14: 259, 1903; 19: 231, 1907.

WEARN, J. T., A. C. ERNSTENE, A. W. BROMER, J. S. BARR, W. J. GERMAN AND L. J. ZSCHIESCHE. This Journal 109: 236, 1934.

FURTHER STUDIES CONCERNING THE OVERT AND MASKED ACTIONS OF STEROIDS

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All hormonally active steroids examined up to the present time possess some degree of folliculoid activity (1–5). It has been postulated that certain common actions of steroids with otherwise vastly different pharmacological properties are due to the fact that they are all endowed with some folliculoid activity. Among the best studied folliculoid effects common to the steroid hormones are the ability to cause at least transitory vaginal cornification, uterine enlargement, mammary gland development, thymus involution, atrophy of the Leydig cells and the spermatogenic epithelium in the testis as well as to prevent the development of castration cells in gonadectomized rats. To mention only the most prominent representatives of the various groups of steroids one may say that these effects are exhibited by estradiol, testosterone, progesterone and desoxycorticosterone, but that the degree of this activity decreases in the order in which the compounds are mentioned. That all these apparently unrelated effects are really subordinate manifestations of a single (namely, the folliculoid) action, received further support by the observation that a striking parallelism exists between the degrees to which these effects are exhibited at the same time by any one compound. This theory has proved practically applicable inasmuch as it has frequently permitted the prediction of the degree to which a compound would exhibit the various folliculoid activities at a time when it had been examined for only one of these manifestations. Thus, for instance, a steroid having a pronounced anti-thymus effect invariably proved to be potent with regard to its anti-Leydig cell action and *vice versa* (6).

It may be asked that if all the above-mentioned effects are merely manifestations of a single pharmacological activity, how is it possible that in the case of certain compounds some actions (*e.g.* vaginal cornification, involution of the spermatogenic epithelium) are not manifest under all experimental conditions. We believe that this is due to the fact that some folliculoid effects are inhibitable and can be masked by other steroid hormone actions. The correctness of this assumption has been proven by actual experimentation since it was shown that the destruction of the spermatogenic epithelium (7) or the cornification of the vagina (8) normally elicited by estradiol or estrone is completely prevented if either progesterone or testosterone is administered simultaneously with the folliculoids. Since even the effects of added pure folliculoids are thus masked by luteoid and testoid compounds, it is hardly surprising that the slight folliculoid activity inherent in the testosterone or progesterone molecule can also be

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[1] Working under a grant from the Banting Research Foundation.

masked in this manner under certain experimental conditions. This further
elaboration of our theory likewise proved of heuristic value inasmuch as it
helped to explain the rather surprising fact that small doses of testosterone cause
testis atrophy, while large doses tend to exhibit the opposite effect. It has been
assumed, in agreement with the above considerations, that the production of
testis atrophy is due to the folliculoid effect of testosterone which endows the
compound with an anti-spermatogenic action. At low dose levels this effect is
overt, while at high dose ranges it is masked by the independent spermatogenic
action of the compound. The fact that depending upon the dose level one or
the other of these two activities prevails, can readily be explained if we assume
that the effective threshold for the spermatogenic action is much higher than
that of the anti-spermatogenic action; hence at low dose levels the former can-
not manifest itself. That this explanation is correct was shown by the adminis-
tration of a mixture containing a constant proportion of Δ^5-androstene-3(β),17
(α)-diol and α-estradiol. The former compound is spermatogenic at all effective
dose levels (apparently because it possesses comparatively slight folliculoid
properties), while the latter substance is, of course, intensely anti-spermatogenic.
As we predicted on the basis of our theory, this mixture proved to cause testis
atrophy at a low, but not at a high dose level (9).

The experiments upon which we should like to report in this communication
have been planned in the hope that our theory might also help to explain the
rather contradictory results recorded in the literature concerning the production
of vaginal cornification in spayed rodents with various androstane and alkyl-
substituted androstane derivatives. With many of these compounds some work-
ers obtained marked vaginal cornification at low dose levels, while others were
unable to confirm such an "estrogenic" effect although much higher doses were
used. It will be recalled that we (8) found that if small doses of estrone are
administered simultaneously with large doses of progesterone the vagina corni-
fying effect of the former is inhibited by the latter and vaginal mucification
results. In these experiments 400 γ of progesterone were required to inhibit
the action of estrone. Subsequent investigators (10–13) essentially confirmed
these observations but the ratio between estrone and progesterone necessary
to inhibit the cornifying effect of the former differs greatly in these publications
and it has not been possible to establish just how much progesterone is needed
to mask this effect of a given dose of a folliculoid. According to our theory this
is understandable since the effect depends not only upon the ratio between the
two steroids, but also upon the dose level tested. At low dose ranges we would
expect the folliculoid and at high dosage levels the luteoid action to prevail.

The following experiment shows that in this case prediction on the basis of
our theory again received support from actual observations.

Twelve female albino rats weighing 160–175 grams were spayed and two days
later divided into two groups of six animals. Group I received a mixture of 0.5
mgm. of progesterone and 25 γ of α-estradiol dissolved in 0.2 cc. of peanut oil
per day. Group II was given 10 mgm. of progesterone and 500 γ of α-estradiol
in 0.2 cc. of peanut oil per day. The daily dose was administered in two subcu-

taneous injections in both these groups, treatment being continued for ten days. The animals were sacrificed twenty-four hours after the last injection. In this manner group II received twenty times as much of the same hormone mixture as was given to group I. All other experimental conditions in the two groups were identical. Daily examination of the vaginal smears throughout the experimental period indicated that all animals of group I showed continuous estrus throughout the experiment, while those of group II revealed only transitory vaginal estrous changes during the first two days. At autopsy the uteri of group I were greatly dilated with fluid exhibiting a typical estrous appearance, while in group II the dilatation was much less evident. Thus it appears that at a low dose level our hormone mixture caused continuous estrus, while this was not the case at the high dose level.

Figure 1 is a schematic drawing which will help to visualize the interaction between the two hormones. The vaginal cornifying effect seen with increasing
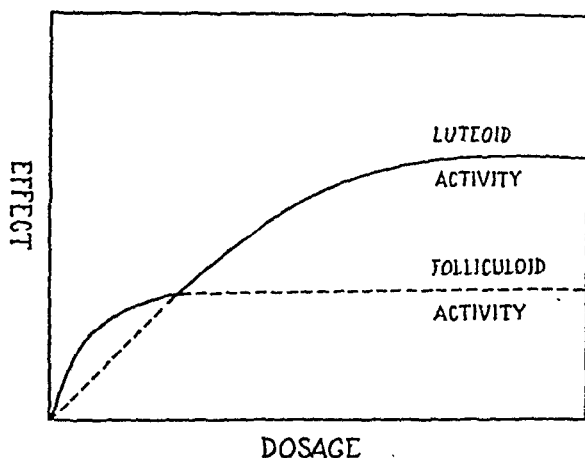
Fig. 1. Diagram showing manifest and masked activities of steroids. Solid line represents manifest and interrupted line masked activities.

doses of estradiol rapidly reaches a maximum above which it is not significantly enhanced by raising the dose. On the other hand the degree of progesterone response rises very slowly but continues to increase over a wider dose range. Hence at low dose levels the folliculoid and at high dose levels the luteoid action will be manifest. It may be said that the transitory vaginal estrus obtainable with all hormonally active steroids can also be explained on the basis of these observations since it must be assumed that the slight folliculoid activity inherent in such compounds as testosterone, progesterone and desoxycorticosterone could only be manifest at very low dose levels in the face of their strong inhibitory actions. Thus signs of vaginal stratification and cornification are detectable only during the first few days of treatment when the hormone concentration in the body is still low. It will be noted that our schematic drawing can equally well be used to illustrate either the effect of a single compound endowed with folliculoid and luteoid activity or of a mixture of a folliculoid and a luteoid compound. Depending upon the relative amounts of these two activities present in

a single steroid or a mixture, the point at which the two curves cross will shift to the left or right and the effective dose range of the folliculoid activity will become smaller or larger respectively.

In conclusion we wish to re-emphasize the broader implications of these observations concerning the interpretation of hormonal actions in general. It has often been claimed that if a crude extract of an endocrine gland can be separated into two fractions with qualitatively different effects, one may assume that two distinct hormones are present which have at least partially been separated. The above observations show that this type of argument is not conclusive since qualitatively different effects (cornification or mucification) may be obtained with a mixture containing a constant proportion of two hormones depending upon the dose level tested. It is essential, therefore, in the case of comparatively impure extracts to test the activity of each fraction over a wide dose range before claiming that a separation into distinct principles has been effected.

SUMMARY

Experiments on spayed rats indicate that a mixture containing a constant proportion of progesterone and α-estradiol causes vaginal cornification at low, but not at high dose levels. The experiments are taken to support the concept according to which the transitory vaginal cornification elicited in the spayed rodent by all hormonally active steroids is due to the fact that these compounds contain a certain amount of folliculoid activity. This is manifest during the first few days of treatment when the hormone concentration in the body is still low but becomes masked when this concentration rises above a certain level.

REFERENCES

(1) CLARKE, E. AND H. SELYE. Am. J. Med. Sci. 204: 401, 1942.
(2) CLARKE, E., S. ALBERT AND H. SELYE. Anat. Record 83: 449, 1942.
(3) CLARKE, E. AND H. SELYE. Fed. Proceedings 1: 14, 1942.
(4) SELYE, H. Endocrinology 30: 437, 1942.
(5) SELYE, H. AND S. ALBERT. J. Pharmacol. and Exper. Therap. 76: 137, 1942.
(6) SELYE, H. Rev. Canad. de Biol. 1: 577, 1942.
(7) ALBERT, S. AND H. SELYE. J. Pharmacol. and Exper. Therap. 75: 308, 1942.
(8) SELYE, H., J. S. L. BROWNE AND J. B. COLLIP. Proc. Soc. exper. Biol. and Med. 34: 198, 1936.
(9) SELYE, H. Endocrinology 32: 116, 1943.
(10) COURRIER, R. ET G. COHEN-SOLAL. C. r. Soc. Biol. 124: 961, 1937.
(11) KORENCHEVSKY, V. AND K. HALL. J. Path. 45: 681, 1937.
(12) FREUD, J. Acta brev. Neerl. 8: 130, 1938.
(13) FREED, S. C. AND S. SOSKIN. Proc. Soc. exper. Biol. and Med. 38: 391, 1938.

RÔLE OF THE RENAL INNERVATION IN RENAL TUBULAR FUNCTION

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The data given here were obtained some time ago (1939) but their publication is considered worthwhile because they are evidently the first definite proof that the renal nerve-supply does not affect renal tubular secretion: at least with regard to water, chloride, and phenol-red.

The antecedant contributions, too numerous to be mentioned here, have shown or have failed to show differences in the rate of excretion of various substances between the denervated and the innervated kidney. In no instance, however, was the rate of filtration known; consequently the amount resorbed or the amount outwardly secreted, as the case may have been, was unknown. With the introduction of the inulin-clearance as a direct measure of glomerular filtration, simultaneously by Shannon (1934) and Richards, Westfall and Bott (1934), it has become possible to find the fraction of material resorbed or outwardly secreted. Incidentally, in the dog, the creatinine-clearance is equal to the inulin-clearance (Shannon, 1935a).

The amount of substance resorbed per unit time can be easily calculated:

$$\text{Amount resorbed} = \text{Amount filtered} - \text{Amount excreted}$$

The amount filtered equals the rate of filtration of plasma multiplied by the concentration of the free substance in the plasma, or, more strictly, in the plasma-water. All the Cl in the plasma is free. The amount of water filtered per unit time is taken equal to the rate of glomerular filtration (=inulin- or creatinine-clearance). This assumes that the volume occupied by the non-filterable constituents of the plasma is negligible.

For a substance which is outwardly secreted:

$$\text{Amount secreted} = \text{Amount excreted} - \text{Amount filtered}$$

Phenol-red is such a substance but the amount of it filtered was not known because the protein-bound fraction was not ascertained. The relative activity of the tubules of both kidneys with regard to PR-secretion could, however, be estimated by comparing the ratios of the inulin- and PR-clearances for both kidneys (table 1).

Chloride, water, and phenol-red were chosen as test-substances because they are easy to measure and are transported by actual activity on the part of the tubules (see Results).

To make sure of complete denervation, the test-kidney was excised and transplanted.

The surgery was performed by Dr. Kenneth W. Thompson, Department of

Surgery, Yale University. To Dr. Robert W. Clarke of the Laboratory of Physiology, Yale University, the writer owes his initiation into the technique of measuring renal clearances and thanks for help throughout.

METHODS. *1. Surgical.* The hound was a young female weighing 15.2 kgm. *Operation 1. Transplantation of right kidney to right femoral region* (Jan. 3, 1939). After all was prepared and the right ureter had been mobilised with plenty of fat to spare its blood-supply, the right renal pedicle was divided close to the aorta and the anastomosis done forthwith. In order to have approximately equal ischemia in both kidneys during the operation, the rubber-clamp on the left renal pedicle was tightened just after the right pedicle was divided. After the anastomosis, the clamp was removed from the femoral artery and the blood was allowed to flow through the kidney for 30 seconds. There was no leak at the junctions. The femoral and renal veins were then anastomosed, after which all clamps were immediately removed. The circulation became re-established apparently satisfactorily. The blood-supply had been obstructed for 25 to 30 minutes. The transplanted kidney was firmly sutured in place by tacking fatty tissue on all sides of it. After the operation 300 cc. saline plus 10 per cent glucose were given intravenously. Postoperative convalescence was uneventful. *Operation 2. Transplantation of central portion of bladder including trigone to ventral abdominal wall* (Feb. 2, 1939). The bladder was developed with special care to preserve the blood-supply of trigone and ureters. Neither ureter was dilated. The bladder was then opened and the distal end of the urethra was ligated. The mucous membrane of the bladder was united to the skin of the abdominal wall. Saline-glucose was again given intravenously and continued subcutaneously a few times during the following week. Convalescence was uneventful. The discharge of urine from the ureteral orifices was rhythmical throughout the period of study.

2. Convalescence and training. During the subsequent 2 days, the bladder and thigh-region were bathed twice daily with 85 per cent ethyl alcohol. Subsequently the bladder was washed twice daily with warm sterile saline and warm saturated boric acid. There were no signs of infection. The dog was constantly vigorous and had a good appetite, normal feces, and a rectal temperature of about 38.5°C. Except for the first day after the operation, it was fed milk and a pound of meat daily. It gradually increased to normal weight from the initial somewhat emaciated condition of January 3.

Tests during the first week after the operation showed the urine to be acid to litmus and alkaline to methyl-orange and methyl-red. There was, however, some pus in the urine from the right ureter; but the urine was never malodorous. A renal antiseptic was administered: 7.5 grains methyl-amine plus 7.5 grains NaH_2PO_4 (as an antibase) were given, with the meat, twice daily. On February 23, the pus was almost negligible and the antiseptic was no longer given. On January 10 glycosuria disappeared but there was always a slight amount of protein in the urine from the right ureter. Preliminary measurements of urinary flow, depression of the freezing-point, chlorine- and urea-excretion gave no evidence of malfunction in either kidney. The concentration of urea in the

blood was normal notwithstanding the meat diet. Phenol-red, injected subcutaneously, appeared simultaneously from both ureters (detected by application of $NaHCO_3$ to the exteriorised bladder).

The dog, which was good-tempered, was trained to lie quietly while strapped horizontally to the dog-board. An anesthetic was never used outside of the surgical operations.

3. Physiological procedure. In some experiments the inulin-clearance was used as a measure of glomerular filtration (table 1). The infusion-solution consisted of 40 grams inulin (Pfanstiehl, c.p., non-pyrogenic to dogs) dissolved at

TABLE 1

Two experiments showing amounts of water and chlorine resorbed per unit volume of glomerular filtrate and the relative potencies of both kidneys to excrete phenol-red

| TIME | URINARY FLOW (cc./min.) | | PLASMA (MGM. PER CENT) | | | URINE (MGM. PER CENT) | | | | RATIO OF CLEARANCES (LEFT/RIGHT) | | MGM. Cl RESORBED/100 CC. GLOM. FILTRATE | | CC. WATER RESORBED/100 CC. GLOM. FILTRATE | | MGM. Cl EXCRETED PER MIN. | |
| | | | | | | Inulin | | PR | | | | | | | | | |
	Left	Right	Inulin	PR	Cl	Left	Right	Left	Right	Inulin	PR	Left	Right	Left	Right	Left	Right
min.																	
	Infusion-expt. with inulin and PR (Feb. 22, 1939)																
−30	About 300 cc. tepid tap-water by gastric tube																
−15	About 250 cc. tepid tap-water by gastric tube																
0	Infusion-solution enters left ext. jug. vein at a little over 5 cc./min.																
10	PR is evident from both ureters simultaneously (NaHCO₃-test)																
20	Rate of infusion lowered to 2.5 cc./min.																
25-37	0.6	1.1	337	1.42	390	9,350	6,500	44.0	33.8	0.79	0.71	384.5	377.6	96.4	94.8	0.87	2.6
43.5-56.5	0.185	0.63	361	1.52	390	14,000	11,750	90.5	74.5	0.32	0.36	383.9	377	97.4	97.2	0.44	2.9
97-110	0.78	1.09	380	1.64	390	17,200	8,250	117.0	58.3	1.49	1.44	385.3	373.5	97.8	95.4	1.71	3.9
	Infusion-expt. with inulin and PR (Mar. 1, 1939)																
−64	About 600 cc. tepid tap-water by gastric tube																
−37	About 500 cc. tepid tap-water by gastric tube																
−18	30 cc. priming solution (10 cc. 0.5 per cent PR; 30 cc. Ringer's; 2 g. inulin) subcutaneously; 15 cc. through each side of lateral abdominal wall																
0	Infusion-solution passes into left ext. jug. vein at ca. 4 cc./min.																
10	Rate of infusion lowered to 2 cc./min.																
19-25	2.58	1.58	170	1.25	355	1,600	1,750	16.9	20.2	1.49	1.37	353.9	352.1	89.3	90.3		
37-42.5	2.49	1.38	229	1.68	357	2,300	2,250	22.7	24.6	1.85	1.67	355	354	90.0	89.8		
71-81	1.44	1.16	294	1.83	364	2,875	2,750	27.6	28.8	1.31	1.19	361.9	360.8	89.8	89.3		
86-94.5	2.38	1.82	315	1.91	363	3,325	3,300	31.0	31.7	1.32	1.28	361.1	361.1	90.5	90.4		
106.5-116	2.3	1.43	336	1.96	363	3,775	3,550	33.7	33.2	1.72	1.64	362.5	361.9	91.1	90.5		

85°C. in mammalian Ringer's; to this was added 40 cc. 0.5 per cent PR. The water had been freshly glass-distilled and the solution was sterilised before use. The solution was passed through the left external jugular vein at 2.5 to 5.0 cc. per minute. In some instances a priming solution was given (table 1, Mar. 1). The urine was diluted immediately after being collected and measured.

Because we were studying tubular activity, the attempt was made to have the plasma-PR close to 1.5 mgm. per 100 cc. (table 1). At this concentration the fraction excreted by secretion approximates 83 per cent, but at a plasma-concentration of about 40 mgm. per 100 cc. only 35 per cent is excreted by secretion (Shannon, 1935b). In other instances the clearance of exogenous creatinine

was taken as the measure of glomerular filtration (table 1). Ten per cent solutions were injected subcutaneously into the lateral abdominal region: one-half of the total quantity into each side, after which the sides were briefly massaged. The creatinine was dissolved first in a minimal quantity of distilled water as it is not soluble in Ringer's to the extent of 10 per cent. The urine was collected into test-tubes by gentle suction (6–7 mm. Hg) applied to both ureteral

TABLE 2

Three experiments showing amounts of water and chlorine resorbed per unit volume of glomerular filtrate

TIME	URINARY FLOW (CC./MIN.)		PLASMA (MGM. PER CENT)		URINE (MGM. PER CENT) Creatinine		MGM. Cl RESORBED/100 CC. GLOM. FILTRATE		CC. WATER RESORBED/100 CC. GLOM. FILTRATE		MGM. Cl EXCRETED PER MIN.	
	Left	Right	Creat.	Cl	Left	Right	Left	Right	Left	Right	Left	Right
min.												
	(April 21)											
−41	25 cc. 10 per cent creatinine in Ringer's, subcutaneously											
−22--−7		0.14	14.5	435		1,431		427.5		99.0		1.04
0	About 900 cc. tepid tap-water by gastric tube											
19–47	0.047	0.127	18.4	407	2,890	2,270			99.4	99.2		
67–76	1.25	0.74	16.8	392	326	356	386.8	387.3	94.8	95.8	1.25	0.73
93–99	1.9	1.0	16.2	392	222	226	388.3	389.2	92.7	92.8	0.95	0.45
	(April 24)											
−54	30 cc. 10 per cent creatinine in Ringer's, subcutaneously											
−21--−6	0.059	0.133	21.5	420	4,320	2,475			99.5	99.1		
0	About 900 cc. tepid tap-water by gastric tube											
22–23	0.105	0.225	24.4	325	5,010	2,250	391.7	390.8	99.5	98.9	0.77	0.88
71–77	2.47	1.18	16.2	388	246	351	385.2	384.9	93.4	95.2	1.04	0.77
95–101	2.04	1.70	16.1	389	227	192	386.9	385.9	92.9	92.0	0.61	0.68
	(May 31)											
0	About 700 cc. tap-water by gastric tube											
25	25 cc. 10 per cent creatinine in Ringer's, subcutaneously											
88–93	3.3	1.8	12.4		147.4	143.2			91.6	91.3		
101–106	4.2	1.7	12.3		144.3	126.0			91.6	90.2		
115–120	3.2	1.7	12.0		179.0	161.0			93.0	92.5		

orifices simultaneously. The glass-tubes applied to the orifices were capillaries enlarged into small cups at one end.

4. Chemical methods. All analyses were made in duplicate. The values were compared with standard solutions. Blood-clotting was prevented by a minute amount of heparin in the needle and nozzle of the syringe.

a. *Urea* was estimated manometrically (Van Slyke, 1927). The urine was diluted 100 times.

b. *Chlorine* in plasma and urine was analyzed by the titrimetric method of Volhard (1878) as modified by Van Slyke and Sendroy, Jr. (1923).

c. *Inulin* in plasma and urine was measured, after yeast-adsorption and acid-

hydrolysis (see Shannon and Smith, 1935), by the Shaffer-Hartmann-Somogyi method (see Shaffer and Somogyi, 1933) using Shaffer-Hartmann reagent "50" and Somogyi's (1931) procedure for deproteinisation of the plasma.

d. *Exogenous + endogenous (?) creatinine* was analyzed by the Jaffé reaction (method of Folin and Wu, 1919; see also Shannon *et al.*, 1932). The plasma was made protein-free by an equal volume of 6 per cent Na_2WO_4 and of $0.33N$ H_2SO_4. The urine was diluted 100 to 3000 times so that differences in the colorimetric readings (Duboscq colorimeter supplied with green Wratten filter) were not over 50 per cent and generally much less than that. In some experiments an Evelyn photoelectric colorimeter was used but no distinct advantage was so gained.

e. *Phenol-red* was measured colorimetrically. One drop of saturated Na_2CO_3 was added to the 2 cc. of plasma or the tenfold diluted urine just before taking a reading. From this reading the value of the plasma, collected before administration of the PR, was subtracted. An ϵ 74 Wratten filter was used with the Duboscq colorimeter (see Shannon, 1935a).

RESULTS. Since the urine of the mammal is ordinarily hypertonic to the blood (Hoppe-Seyler, 1859), the tubular resorption of water beyond the stage producing isosmoticity with the blood must be an active process. Even in ordinary diuresis, the mammalian urine is markedly hypertonic. Furthermore, when the Cl-concentration of the blood falls, the Cl-concentration of the urine may fall, not only well below the concentration of Cl in the plasma (see tables 1 and 2), but almost to the vanishing point. Clearly, Cl must be actively resorbed by the tubules. Lastly, the fact that the PR-clearance invariably exceeds the inulin-clearance (table 2) shows that PR is secreted outwardly by the tubules.

Inspection of tables 1 and 2 shows that the amount of water and chlorine resorbed *per unit volume of glomerular filtrate* is identical in the transplanted and in the non-transplanted kidney. This is true even when a large fraction of this resorption is the result of tubular activity. Furthermore, the ratios of the inulin- and PR-clearances are practically identical for both kidneys (table 1) showing that the fraction of phenol-red outwardly secreted by the tubules of both kidneys is the same. These data demonstrate, conclusively in our opinion, that the renal nerve-supply has no influence on renal tubular activity, at least with regard to water, chloride, and a foreign substance such as phenol-red. Because the active transport of substances by the tubules follows the mass-law—at least with regard to substances which have been most extensively studied with this respect (Shannon, 1939)—one would not expect, even *a priori*, the renal nerves to exert an influence on tubular secretion.

On the other hand, our data show, as do those of many of our predecessors, that the rate of urinary flow and of the excretion of chloride is not necessarily identical from both kidneys. As shown in this paper, however, such differences do not reside in dissimilarities in tubular function and must, therefore, be due to differences in glomerular activity. We do not, however, imply that changes in glomerular activity are necessarily concomitant with renal denervation.

In the above experiments the animal was not anesthetised. It was so used

to being strapped on the dog-board and having an infusion-needle in its external jugular vein that it frequently yawned during the procedure.

SUMMARY AND CONCLUSION

1. A dog's kidney was successfully transplanted to the femoral region by end-to-end anastomosis of the renal vessels with the femoral; the bladder and ureteral orifices were exteriorised.

2. The amounts of water and of chlorine resorbed per unit volume of glomerular filtrate (inulin- or creatinine-clearances) were identical in both the transplanted and non-transplanted kidney. This was true both during antidiuretic and diuretic urinary flows.

3. The ratios of the inulin- and phenol-red clearances were practically identical for both kidneys, showing that the fraction of phenol-red outwardly secreted by the tubules of both kidneys was the same.

4. Our data demonstrate that any differences in the rate of excretion of water and of various solutes by the normal and fully denervated kidney are not due to dissimilaritites in tubular function and hence must be due to differences in glomerular circulation.

REFERENCES

FOLIN, O. AND H. WU.   J. Biol. Chem. **38**: 81, 1919.

HOPPE-SEYLER, F.   R. Virchow's Arch. **16**: 412, 1859.

RICHARDS, A. N., B. B. WESTFALL AND P. A. BOTT.   Proc. Soc. exper. Biol. and Med. **32**: 73, 1934.

SHAFFER, P. A. AND M. SOMOGYI.   J. Biol. Chem. **100**: 695, 1933.

SHANNON, J. A.   J. cell. comp. Physiol. **5**: 301, 1934.
    This Journal **112**: 405, 1935a.
    Ibid. **113**: 602, 1935b.
    Physiol. Rev. **19**: 63, 1939.

SHANNON, J. A., N. JOLLIFFE AND H. W. SMITH.   This Journal **102**: 534, 1932.

SHANNON, J. A. AND H. W. SMITH.   J. Clin. Investigation **14**: 393, 1935.

SOMOGYI, M.   J. Biol. Chem. **90**: 725, 1931.

VAN SLYKE, D. D.   Ibid. **73**: 695, 1927.

VAN SLYKE, D. D. AND J. SENDROY, JR.   Ibid. **58**: 523, 1923.

VOLHARD, J.   Ztschr. anal. Chem. **17**: 482, 1878.

A DIURNAL RHYTHM IN THE BLOOD SUGAR
OF THE WHITE RAT

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It has been observed by Cori and Cori (2) that the concentration of blood sugar is an important factor determining the rate of glycogen formation in the liver. We also note that those investigators who have more recently studied diurnal variations of liver glycogen in the white rat (3, 7, 8) present convincing evidence that a definite diurnal rhythm of glycogen deposition occurs in this animal and that this rhythm is due to cyclic feeding habits. The natural corollary to these two observations is that the white rat should show a diurnal rhythm also in blood sugar concentration, and that this rhythm, like that of liver glycogen, should be determined by cyclic feeding habits. The purpose of the investigation reported here was to test this hypothesis.

Aside from clinical observations on the diabetic human being studies on diurnal variations of blood sugar concentration are rare. The few authors who have attacked the problem have worked on a variety of animals with a consequent variety of results. Krasnjanskij (10) working on normal human subjects found a sharp rise in blood sugar following each meal. However, his data give no suggestion of any general diurnal trend. Allcroft (1) found a different situation in the lactating cow. He was able to demonstrate a diurnal rhythm with the maximum blood sugar concentration occurring at 3 to 4 a.m. and the minimum at 8 to 9 a.m. He was unable to find any such rhythm in the dry cow or sheep. Euler and Holmquist (4) working on the rabbit, found a definite blood sugar rhythm with the maximum occurring between 4 and 10 a.m. Still later Jores (9) working on ten normal human subjects disagreed with Krasnjanskij to the extent that he was able to demonstrate a diurnal rhythm in the blood sugar with the maximum concentration occurring at 4 a.m. and the minimum at 3 to 4 p.m.

METHODS. Virgin female rats four to six months of age and 175 to 250 grams weight were used in all experiments. In the present work a certain importance is attached to the diurnal spontaneous activity rhythms of the animals used and the fact that the spontaneous activity rhythm of the female rat is clearer and less variable than that of the male accounts for the exclusive use of females in these experiments. It may be pointed out, however, that experiments on male rats indicate that similar results are obtained with both sexes. The rats were kept in wire cages with activity recorders of the type illustrated and described by Griffith and Farris (5) and were exposed to normal daylight and darkness. Water was always available and Purina rat food was used consistently.

The blood sugar concentrations were determined by the microtitration method

of Miller and Van Slyke (11). This method gives essentially the fermentable sugar thus yielding values lower than those obtained by methods which determine total reducing substances. The blood was obtained by clipping the tip of the animal's tail with a pair of scissors. Three or four drops were allowed to fall into a waxed dish containing powdered sodium citrate to prevent coagulation. From this dish the 0.1 cc. required was pipetted out and precipitated in cadmium sulfate solution. It was observed that blood sugar values were abnormally high when more than three minutes elapsed between the time an animal was picked up and the time the last drop was obtained from the tip of its tail. This was probably an excitation effect akin to Cannon's "emergency hyperglycemia." Consequently, all the determinations used in this paper are based on blood samples obtained in less than three minutes.

All blood samples were taken at 12 noon and 12 midnight (EST) ± 45 minutes. It was observed that when a rat was bled at twelve hour intervals for a number of days, the blood sugar values obtained from the later bleedings frequently showed great variability, deviating markedly from the pattern obtained in the first few bleedings. A possible explanation of this is that frequent clipping of the tail caused painful swelling, a disturbing condition which was reflected in the blood sugar. Consequently, the rats used in these experiments were never bled more than three consecutive times at twelve hour intervals.

PROCEDURE AND RESULTS. The first point to be investigated was whether or not the blood sugar of the normal white rat, feeding freely, exhibits a diurnal rhythm. This was investigated by the simple procedure of making a series of blood sugar determinations on blood samples taken from such rats at both noon and midnight. In this experiment fifty-four determinations were made on eleven different animals. The animals were allowed a three to five day recovery period after each set of three consecutive bleedings before being used again. Half of the bleedings were in the order noon-midnight-noon while the other half were made in the opposite order, midnight-noon-midnight. This procedure was followed in all the experiments described and was intended to eliminate the possibility that the observed changes in blood sugar concentration might be a direct response to the experimental technique used. A true diurnal rhythm obviously would give reversed patterns under the two different procedures whereas a direct response to the technique of bleeding would be the same in both cases.

The results of these experiments are given in table 1. The data in this table make it obvious that the blood sugar concentration of normal animals is approximately 10 per cent higher at midnight than at noon. The usual criterion for statistical significance, i.e., that the difference between the mean midnight value and the mean noon value must be more than twice its standard error, is used throughout this paper. In this case the difference between the means is more than six times its standard error.

Having established the presence of a diurnal blood sugar rhythm by this first group of experiments on animals feeding freely, it then became desirable to know to what extent this rhythm was affected by the animals' cyclic feeding

habits. Two procedures were used to obtain this information, i.e., fasting and enforced reversal of the animal's feeding cycle. The fasting studies were made upon two groups of six rats each. They were subjected to the following fasts.

Group I: a. Thirty-six hour fast beginning at midnight, the first blood sample being taken at noon twelve hours after the removal of food.

b. Forty-eight hour fast beginning at noon, the first blood sample being taken at noon twenty-four hours after the removal of food.

c. Forty-eight hour fast beginning at midnight, the first blood sample being taken at midnight twenty-four hours after the removal of food.

TABLE 1

Determinations on rats allowed to feed freely

VALUES	NUMBER OF ANIMAL										
	14	15	16	17	18	19	20	21	22	23	25
mgm. per cent											
Day	96	93	98	96	99	94	99	89	94	99	97
	97	97	99	88	97	93	101	90	88	97	106
					92			99		92	
					97					100	
					100					94	
					102						
Night	104	113	123	98	105	104	109	103	103	108	119
	105	96	107	99	106			110		104	
					103					97	
					104					106	
					104						
					110						

	Standard deviation	Mean	Standard error of mean
Day values.....................................	4.24	96.1	0.78
Night values...................................	6.23	105.9	1.27
Difference between means.......................	....	9.8	
Standard error of difference between means......................			1.49

Group II: a. Fast corresponding to c above.

b. Sixty hour fast beginning at midnight, the first blood sample being taken at noon thirty-six hours after the removal of food.

The statistical summaries of the results of these fasting experiments are to be found in part I of table 2. A study of the table makes it clear that the diurnal rhythm in blood sugar concentration persists during as much as forty-eight hours of fasting but disappears in fasts longer than this. As the length of the period of fasting is increased, the curve of diurnal variation tends to flatten out. After about forty-eight hours of fasting it levels off at approximately 80 mgm. per cent which is roughly 10 mgm. per cent below the average noon figure for animals allowed to feed freely.

It is of interest to note that in the first group of animals a fast of forty-eight

TABLE 2

Statistical summaries of experiments involving fasting and reversal of feeding cycle

CONDITIONS OF EXPERIMENT	TIME OF BLEED-ING	STAND-ARD DEVIA-TION	MEANS	ST. ERROR OF MEANS	ST. ERROR OF DIFFER-ENCE BETWEEN MEANS	IS THE DIF-FERENCE SIGNIFI-CANT?
			mgm. per cent			
I. Fasting experiments:						
A. Group I						
1. Thirty-six hour fast beginning at midnight, the first blood sample being taken at noon 12 hrs. after food removal	N* M*	4:04 6.88	79.8 85.1 ——— 5.3†	1.17 2.81	1.49	Yes
2. Forty-eight hour fast beginning at noon, the first blood sample taken at noon 24 hrs. after food removal	N M	5.34 9.64	78.7 90.2 ——— 11.5†	1.88 4.31	4.71	Yes
3. Forty-eight hour fast beginning at midnight, the first blood sample being taken at midnight 24 hrs. after food removal	N M	5.34 8.40	86.0 84.8 ——— 1.2*†	2.39 2.66	3.58	No
B. Group II						
1. Fast corresponding to "3" of group I (fig. 1)	N M	5.64 7.27	86.8 99.0 ——— 12.2†	2.30 3.46	4.16	Yes
2. Sixty hour fast beginning at midnight, the first blood sample being taken at noon 36 hrs. after food removal (fig. 2)	N M	6.90 3.65	76.4 79.8 ——— 3.4†	1.99 1.49	2.48	No
II. Experiments on rats allowed to feed only from 9 a.m. to 5 p.m.						
A. Determinations on the 5th and 6th days of the new feeding regimen (fig. 3)	N M	5.71 5.40	94.8 102.0 ——— 7.2†	1.80 2.23	2.86	Yes
B. Determinations on 14th to 15th days of the new feeding regimen (fig. 4)	N M	3.94 4.75	105.3 95.0 ——— 10.3†	1.61 1.37	2.12	Yes
C. Determinations on the 20th and 21st days of the new feeding regimen	N M	6.36 6.57	109.8 97.3 ——— 12.5†	1.76 2.49	3.05	Yes

* N = noon, M = midnight.

† Difference.

hours beginning at noon did not abolish the diurnal rhythm whereas a forty-eight hour fast beginning at midnight did so. A possible explanation of this is that since rats normally do not begin to feed until 6 or 7 p.m., a fast beginning at noon does not become effective until six or seven hours later, whereas one beginning at midnight becomes effective immediately. Hence, a forty-eight hour fast beginning at noon is, physiologically speaking, only a forty-one or forty-two hour fast. It is also to be noted that the diurnal rhythm was more easily abolished in the first group of animals than in the second, a sixty hour fast being required in the latter case. The only notable difference between the two groups is that the rats of the first group (18 to 21, 23 and 25) were from one to one and a half months older than those of the second group and had been
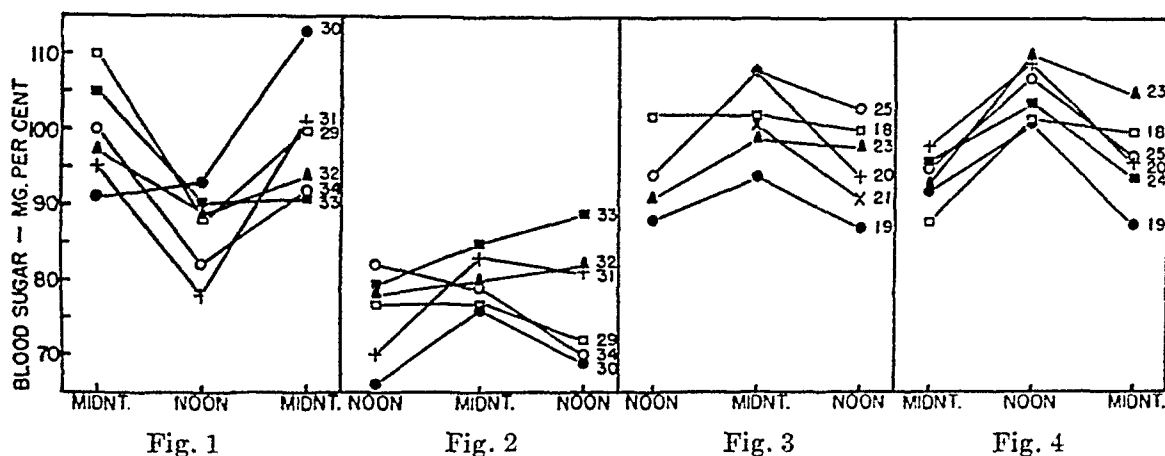
Fig. 1. Determinations on rats which fasted a total of 48 hours. Food was removed 24 hours before the first midnight determinations. These rats were of group II.

Fig. 2. Determinations on rats which fasted a total of 60 hours. Food was removed 36 hours before the first noon determinations. These rats were of group II.

Fig. 3. Determinations on the 5th and 6th days of the new feeding regimen. These animals were forced to feed between the hours of 9 a.m. and 5 p.m.

Fig. 4. Determinations on the 14th and 15th days of the new feeding regimen.

The figures in the right hand margin of each frame designate the number of the animal represented by the adjacent symbol.

subjected to experimental procedures for a longer period of time. One might expect that the diurnal rhythm in blood sugar concentration would be stronger and less variable in a group of young rats which had led an undisturbed existence prior to the fast than in an older group of rats which had been subjected to a number of previous bleedings and fasts. This is a possible explanation of the results obtained.

The data on animals 29 to 34 are graphically shown in figures 1 and 2, figure 1 being based on the data obtained during the forty-eight hour fast and figure 2 on the data obtained during the sixty-hour fast. We observe that in both cases the animals show greater variability than is shown by animals allowed to feed ad lib. However, in figure 1 the diurnal rhythm is still obvious in most most cases while in figure 2 it is obliterated in all animals except no. 30 and possibly no. 31.

In the last group of experiments rats were taught to change their usual feeding habits in an attempt to determine whether this would be accompanied by a similar change in their blood sugar cycle. Seven animals were allowed to feed only between the hours of 9 a.m. and 5 p.m. rather than during the hours of darkness. However, their period of maximum spontaneous activity still occurred during the night. This constituted a separation of the feeding and activity cycles. When these animals had completed adaptation to the new feeding regimen (a period of forty-eight to seventy-two hours as indicated by eventual maintenance of, or increase in, body weight) they were subjected to three sets of bleedings, the first beginning on the fifth day after initiation of the new feeding regimen, the second on the fourteenth day, and the third on the twentieth day.

The results of these experiments are summarized in part II of table 2. After five days on the new feeding regimen the blood sugar cycle was essentially unchanged (fig. 3). However, after fourteen days the blood sugar cycle was completely reversed (fig. 4), the noon values being approximately 10 per cent higher than the midnight ones, and the difference between the means being even more highly significant than that obtained on the fifth day. The results obtained on the twentieth day support those obtained on the fourteenth day. However, on the twentieth day the difference between the means is even greater. Obviously the normal blood sugar cycle persists for at least five days during enforced day feeding, but becomes completely reversed some time before the fourteenth day.

It is to be stressed that the typical spontaneous activity cycle of the animals persisted during these experiments. In most cases the total daily activity decreased 25 to 50 per cent. But its distribution over the twenty-four hours was essentially unchanged, the period of maximum activity still occurring during the hours of darkness. Hence the blood sugar cycle of the white rat accompanies the feeding cycle rather than the activity cycle.

DISCUSSION. The foregoing data make it obvious that there is a diurnal rhythm in the blood sugar concentration of the normal albino rat allowed to feed ad lib. However, it is to be made clear that these data yield little information as to the exact nature of the diurnal curve. Midnight values may not represent the maxima or noon values the minima of the diurnal curve. But since Deuel and collaborators (3) found the digestive tract of the female rat to contain most food at 12 midnight, one at least has reason to believe that midnight determinations fall closer to the maximum point than to the point of inflection.

The observation of Cori and Cori (2) that the blood sugar concentration is an important factor determining the rate of liver glycogen formation would lead one to expect that the liver glycogen cycle would persist during fasting as long as the blood sugar cycle persists. This is apparently the case. Higgins, Berkson and Flock (7) found that the liver glycogen cycle of the white rat persists for one day after the removal of food but that during the second day it remains constant at a low level. This checks reasonably well with the present

experiments. On fasting rats, blood samples had to be taken within twenty-four to thirty-six hours after food removal (not counting the first six hours of a fast beginning at noon, for reasons explained above) in order to obtain a significant difference between day and night values.

Diurnal rhythmicity in the blood sugar concentration of an animal is most probably evoked by one or more of three principal factors, i.e., periodic changes in light (day and night), periodic habits in activity, and periodic habits in feeding. The first two of these factors are closely interrelated and were not altered at any point in these experiments. However, the third factor was isolated from the first two by the expedient of training the animals to feed during the daylight hours while the lighting conditions and spontaneous activity cycle were unaltered. Since it is possible completely to reverse the blood sugar cycle by such a procedure, one may say that cyclic feeding habits constitute the principal factor determining the diurnal rhythm in blood sugar of the rat.

The reversal of the blood sugar cycle obtained upon reversing feeding habits might have been predicted on the basis of the results of Higgins, Berkson and Flock (8). These workers found that by changing the hours of feeding they were able to shift the liver glycogen cycle of the white rat by six hours. It is of interest, however, that the reversal of the blood sugar cycle is not immediate but involves a "conditioning period" of more than five days. This is reminiscent of the seven to eight day conditioning period found by Hemmingsen and Krarup (6) to be necessary for a complete reversal of the activity cycle of the rat in response to a reversal of the periods of light and darkness. In each case the rhythm to be reversed is so completely integrated with the animal's general physiological "timing" that a reversal is obtained only gradually over a period of days even after the factor directly responsible for the cycle has been drastically altered.

It is noteworthy, finally, that the diurnal variations found to occur in the rat's blood sugar are not incompatible with findings on the human being. Man eats three meals a day and it is known (10) that his blood sugar shows three corresponding rises, one after each meal. The rat, by comparison, may be regarded as an animal which eats only one large meal a day (3), and consequently its blood sugar concentration shows one large increase each twenty-four hours.

SUMMARY

There is a diurnal rhythm in the blood sugar level of the normal, fed, female white rat. The difference between the noon and midnight values is statistically significant and is approximately 10 per cent. This blood sugar cycle persists during thirty-six to forty-eight hours of fasting but disappears during longer fasts.

By training animals to feed during the day it is possible to dissociate the feeding and activity cycles. In such a situation the blood sugar cycle is found to accompany the feeding rather than the activity cycle. However, a conditioning period of more than five and less than fourteen days is involved before the change in the blood sugar cycle is completed.

We may conclude that the diurnal cycle in the blood sugar of the white rat is due principally to cyclic feeding habits.

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REFERENCES

(1) ALLCROFT, W. M.   Biochem. J. 27: 1820, 1933.
(2) CORI, C. F. AND G. T. CORI.   J. Biol. Chem. 85: 275, 1929–30.
(3) DEUEL, H. J., J. S. BUTTS, L. F. HALLMAN, S. MURRAY AND H. BLUNDEN.   J. Biol. Chem. 123: 257, 1938.
(4) EULER, U. S. AND A. HOLMQUIST.   Pflüger's Arch. 234: 210, 1934.
(5) GRIFFITH, J. Q. AND E. J. FARRIS.   The rat in laboratory investigation.   J. B. Lippincott Co., 1942.
(6) HEMMINGSEN, A. M. AND N. B. KRARUP.   Det. Kgl. Danske. Videnskabernes Selskab., Biologiske Meddelelser 13: 7, 1937.
(7) HIGGINS, G. M., J. BERKSON AND E. FLOCK.   This Journal 102: 673, 1932.
(8) HIGGINS, G. M., J. BERKSON AND E. FLOCK.   This Journal 105: 177, 1933.
(9) JORES, A.   Med. Klin. 1: 468, 1934.
(10) KRASNJANSKIJ, L. M.   Biochem. Ztschr. 205: 180, 1929.
(11) MILLER, B. F. AND D. D. VAN SLYKE.   J. Biol. Chem. 114: 583, 1936.

A BLOOD VESSEL DEFECT IN SWINE SUFFERING FROM AN INHERITED BLEEDING DISEASE[1]

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Previous studies on an inherited bleeding disease in a strain of swine owned by the Missouri Agricultural Experiment Station show that the disease is transmitted by both sexes as a Mendelian recessive (1), and that the bleeders have a blood coagulation defect (2, 3).

We have shown more recently that the animals also have a very prolonged saline bleeding time (4), and an abnormally low capillary resistance (5). Neither of these symptoms can be readily attributed to the prolonged coagulation time (2, 3), and in this paper we wish to present evidence for a second abnormality, the improper functioning of injured blood vessels.

EXPERIMENTAL.[2] In studies on the prolonged saline bleeding time of bleeder swine (4), we often observed poor correlation between bleeding times and coagulation times. The coagulation times, obtained at the same time as the bleeding times (4), are shown for the first time in figure 1 of this paper. In all of the studies reported here, we used the following modification of the Lee-White (6) clotting time method: A vaseline-coated 19 gauge Wassermann needle is inserted into the marginal vein of the shaved ear, and 2 cc. portions of blood are collected successively in 4 paraffin-coated test tubes (inside diameter, $\frac{5}{8}$ inch, length, 6 inches). A blood flow of 4–8 cc. per minute is acceptable. The tubes are corked and placed in a bath at 37°. The first tube obtained is tilted at minute intervals until the blood is completely clotted. This procedure is repeated successively on the second, third and fourth tubes. The longest clotting time observed (usually tube 4) is recorded as the whole blood coagulation time. In figure 1, the determinations were carried out at approximately weekly intervals for a period of 3 months.

Figure 1 shows that the whole blood coagulation time is in the range of 36–104 minutes in the bleeders, and in the range of 19–53 minutes in the normal animals. These variations cannot be explained by differences in technique; therefore they must be due to actual variations in blood coagulability. There is, then, a natural 2 to 3-fold variation in the clotting time of both normal and bleeder animals, and some overlapping of clotting time values in the 2 groups. The average

clotting time (fig. 1) of the 3 bleeders for the 92-day experimental period is 67 minutes, and of the 5 normal animals, 31 minutes, a $2\frac{1}{6}$-fold difference. No differences between bleeder and normal were observed with respect to the quality and the firmness of the clotted blood, or its speed of retraction.
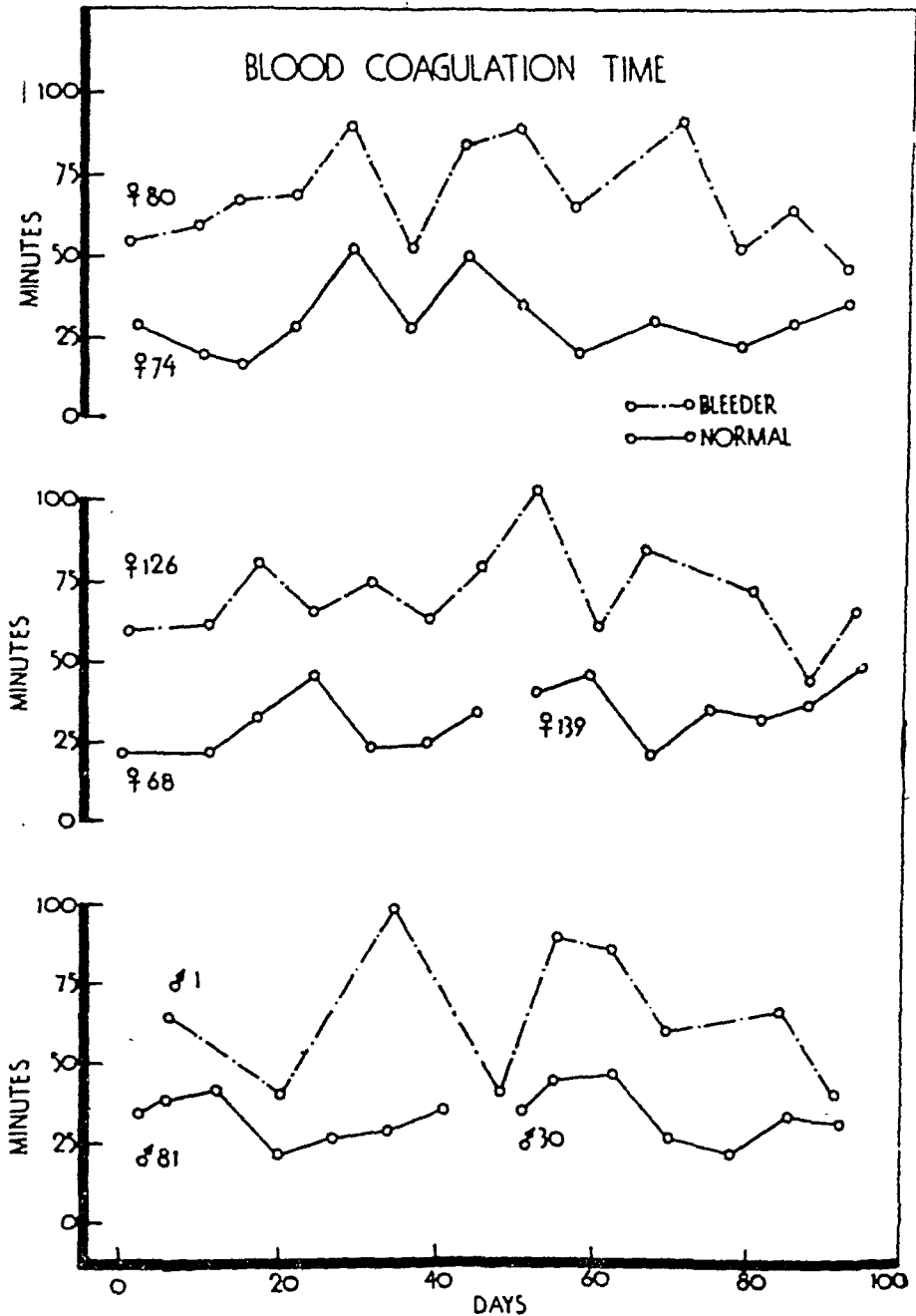
Fig. 1. A comparison of the whole blood coagulation times of bleeder and of normal swine

A comparison of coagulation times (fig. 1) with the saline bleeding times simultaneously obtained (see fig. 1, reference (4)), shows that in several cases (table 1) the coagulation time of the bleeders is short when the saline bleeding time is very prolonged, and vice versa. The anomaly of a short coagulation time

and a long bleeding time was also observed during the routine testing of a group of 4 month old bleeders (bottom of table 1). If the saline bleeding time were dependent on the whole blood coagulation time, one would expect a direct correlation, instead of the values shown in table 1.

We have been able to produce experimentally the combination of a prolonged saline bleeding time and a short coagulation time in bleeders. The data in table 2 show that small whole blood transfusions, while having no effect on the prolonged saline bleeding time, reduce the prolonged coagulation time to within the normal range for several hours.

Furthermore, we have been able to produce experimentally the reverse combination in normal animals, namely, a normal saline bleeding time in the presence of a very prolonged whole blood coagulation time. This is accomplished

TABLE 1

Simultaneous coagulation times and saline bleeding times in bleeders

ANIMAL	DAY (FIG. 1)	COAGULATION TIME	SALINE BLEEDING TIME
		min.	*sec.*
F80	92	48	600+
F126	87	44	550
M1	20	40	490
	91	36	600+
F80	71	90	360
F126	45	79	160
M1	34	99	260
	62	80	220
F7	—	39	600+
M34	—	45	600+
F6	—	42	600+

by administering heparin intravenously. Table 3 shows that the whole blood coagulation time of normal swine can be prolonged 2 to 20-fold by this treatment without affecting the short saline bleeding times of these animals. The coagulation values obtained in the normal swine are much longer than any observed to date in the bleeders (cf. fig. 1).

It is evident that the prolonged saline bleeding time of bleeders can be attributed neither to the prolonged coagulation time which exists simultaneously, nor to a defect in the quality or retraction properties of the bleeder clot. That it is an important symptom of the disease is shown by previous tests on 4–18 month old swine (4), and by the tests on one day old swine reported in table 4. On the basis of inheritance (1), the litter in table 4 should consist of 6 bleeders and 6 carriers, a ratio with which the bleeding time findings closely agree. It is apparent that a prolonged saline bleeding time exists in bleeders *even at birth*.

The relative independence of the saline bleeding time and the coagulation time in swine can be most readily explained by assuming that the 2 tests measure different phenomena. Since the saline bleeding time is not greatly changed by

fluctuations in the coagulability of the blood (tables 1, 2 and 3), it must be governed by changes outside of the blood, i.e., *changes in the blood vessel*. We therefore conclude that the saline bleeding time measures the *constricting ability* of

TABLE 2

The effect of blood transfusions on the saline and coagulation time of bleeders

ANIMAL	TREATMENT	BEFORE TREATMENT		AFTER TREATMENT		
		Saline time	Coagulation time	Hours after	Saline time	Coagulation time
		sec.	*min.*		*sec.*	*min.*
F10	2.2 cc. citrated blood* intrav. per kgm. body wt.	600+	89	2	600+	41
				4	600+	44
				6	600+	52
F6	Same as for F10	600+	88	3	600+	38
				5	600+	61

* An 18 gauge hypodermic needle was inserted into the marginal ear vein of a normal hog, and 4 parts of blood were allowed to flow from the needle into 1 part of 3.8 per cent sodium citrate solution.

TABLE 3

The effect of heparin on the coagulation time and saline bleeding time of normal swine

ANIMAL	TREATMENT	BEFORE TREATMENT		AFTER TREATMENT	
		Coagulation time	Bleeding time	Coagulation time	Bleeding time
		min.	*sec.*	*min.*	*sec.*
F213	0.5 mgm. Na heparin* intrav./ kgm. body wt.	33	85; 85	660+ (5 min. after) 390 (19 min. after)	90 (7 min. after) 90 (17 min. after)
M159	0.3 mgm. Na heparin intrav./ kgm. body wt.	33	65; 140	382 (5 min. after) 165 (17 min. after)	93 (7 min. after) 137 (14 min. after)
F76	0.2 mgm. Na heparin intrav./ kgm. body wt.	53	125; 93	229 (5 min. after) 102 (23 min. after)	58 (14 min. after) 140 (17 min. after)

* One milligram Na heparin = 110 "Toronto units."

the blood vessels injured in the test, and that a prolonged saline bleeding time of the type found in bleeders is due to a *failure of the injured vessels to constrict normally*.

Evidence that an induced constriction of the blood vessels has a marked effect in shortening the prolonged saline bleeding time of bleeders is shown in table 5.

Small quantities of 1:1000 adrenaline (Parke, Davis) were injected with a 26 gauge needle into the tip of the shaved ear, and a lancet wound was made immediately about 5 mm. from the site of the injection. The wound was immersed in isotonic saline at 37° and the saline bleeding time was determined in the usual manner (4). Chester White-Poland China crosses, and purebred Poland Chinas were tested. The 3 day old animals shown in table 5 are from the same litter (table 4).

TABLE 4

Saline bleeding time tests on a litter of Chester White-Poland China swine one day of age

BLEEDERS	SALINE TIME	CARRIERS	SALINE TIME
	sec.		*sec.*
M36	600+	M34	60
M30	600+	M31	75
F30	600+	F31	85
F33	600+	F35	65
M35	600+	F34	55
F37	460		
F36	400		

TABLE 5

The effect of adrenaline on the prolonged saline bleeding time of bleeders

ANIMAL	F1	F9	M5	F30	M30	F33	F36	M35	M4	F10	F7
Skin color (W-white; B-black)	W	W	W	W	W	B	B	B	B	B	B
Breed (C-cross; P-pure)	C	C	C	C	C	C	C	C	C	P	P
Age (days)	156	200	40	3	3	3	3	3	38	463	463
Cc. adrenaline (1:1000) injected	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05	0.05
Saline time before injection (sec.)	600+	600+	260	600+	270	375	360	600+	600+	420+	600+
Saline time after injection (sec.)	98	20	60	115	107	560	330	480	600+	600+	600+

It is evident from table 5 that Chester White-Poland China bleeders with white skins show a reduction of the prolonged saline bleeding time to normal after the injection of adrenaline. Because of this hemostatic effect, adrenaline was found to be very useful in the treatment of surface hemorrhages in these animals. Thus, a tooth socket hemorrhage in F1 (table 5) was stopped at once by the injection of 0.1 cc. of 1:1000 adrenaline into the gum adjacent to the bleeding site. White F9 incurred a deep cut on the edge of the snout which bled for more than 2 hours. The injection of 0.05 cc. of 1:1000 adrenaline on each side of the cut

stopped the bleeding immediately; there was no recurrence of the hemorrhage in spite of pronounced vessel dilatation 20 minutes later. This same animal bled for more than 5 hours from a deep cut in the floor of its mouth. Injection of 0.05 cc. of 1:1000 adrenaline on each side of the cut also stopped this hemorrhage in a few minutes, with no recurrence during subsequent dilatation of the affected vessels.

We are at present unable to explain why injected adrenaline has no effect (table 5) on the prolonged saline bleeding time of black-skinned Chester White-Poland China bleeders, and black-skinned purebred Poland China bleeders. In addition, injected adrenaline has no effect in controlling hemorrhage in these animals. The cause of this anomaly may lie in hereditary factors, i.e., a dominance of the black Poland China bleeder strain in the black bleeders, and a dominance of the Chester White normal strain in the white bleeders.

DISCUSSION. The data presented above are in good agreement with the "two-stage" hemostasis theory recently proposed by Macfarlane (7). According to Macfarlane, the first stage of hemostasis in the smaller blood vessels of mammals consists of a temporary constriction of the wall of the severed or otherwise injured vessel; this slows down or stops the loss of blood. The second stage consists of a firm coagulation of the blood and a retraction of the blood clot in the lumen near the temporarily constricted part of the injured vessel. Following this second stage, dilatation of the vessel occurs, but the plugged condition of the injured area prevents further loss of blood. Our findings indicate that both the first and second stages of hemostasis are abnormal in the bleeder swine. Furthermore, our tests with adrenaline show that the type of vessel defect is not exactly the same in the white-skinned and the black-skinned bleeders.

SUMMARY

Evidence is presented to show that swine suffering from an inherited bleeding disease have a blood vessel defect in addition to the previously observed coagulation defect.

REFERENCES

(1) BOGART, R. AND M. E. MUHRER. J. Hered. 33: 59, 1942.
(2) HOGAN, A. G., M. E. MUHRER AND R. BOGART. Proc. Soc. Exper. Biol. and Med. 48: 217, 1941.
(3) MUHRER, M. E., A. G. HOGAN AND R. BOGART. This Journal 136: 355, 1942.
(4) MERTZ, E. T. This Journal 136: 360, 1942.
(5) MERTZ, E. T. This Journal 138: 136, 1942.
(6) LEE, R. I. AND P. D. WHITE. Am. J. Med. Sci. 145: 495, 1913.
(7) MACFARLANE, R. G. Quart. J. Med. 10: 1, 1941.

CIRCULATORY REACTIONS OF RATS TRAUMATIZED IN THE NOBLE-COLLIP DRUM

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These investigations were undertaken during the summer of 1941 and at intervals through the following year to obtain data supplementary to those made by Noble and Collip. Some of the experiments are confirmatory of those published by Noble and Collip (1, 2). In addition, a detailed study was made of blood pressure changes, hematocrit, and plasma volumes, also observations on the visceral and peripheral capillary circulation.

In the course of the observations, it was soon realized that the trauma induced by drumming was very general and caused much congestion, especially in the gut. This made it difficult to differentiate between direct and indirect effects of the trauma on the capillary circulation. Hence, the observations described in this paper on the capillaries are not to be considered as strictly referable to the effects of traumatic shock on capillaries remote from the trauma.

METHODS. Rats, with their paws taped to render them helpless, were placed in a drum, 16 inches in diameter and 7 inches deep, and the drum rotated at a speed of 45–50 revolutions per minute. Two 2-inch shelves, fastened on opposite sides on the inner circumference of the drum, carried the rat with each revolution part way up the circumference until it slipped off and fell. This procedure caused the rat to sustain at least one and more often two falls per revolution thereby undergoing cumulative trauma with the successive falls. It may be stated that the rats gave no sign of pain. They were evidently thrown into a semiconscious state at the first fall, and the successive falls were sufficiently rapid in sequence to prevent recovery until they were removed from the drum.

The experimental data of Noble and Collip show that rats, weighing about 150 grams and subjected to 800 revolutions, underwent injuries with a 100 per cent mortality, while of those subjected to 300 turns the mortality was 8 per cent. In the intermediate range of 600 revolutions the mortality was approximately 75 per cent. The period of survival of the fatal cases, subjected to this intermediate range of trauma, varied from 20 minutes to 10 hours, the great majority (ca 70 per cent) dying within 45 to 90 minutes. These results, indicating the statistically consistent type of trauma involved, have been confirmed by us except for a slightly longer survival period in the 650 revolution range (60–120 min.) in experiments on over 800 rats.

About 500 rats were subjected to 650 revolutions of the drum. A certain percentage of these (10–15 per cent) either died in the drum, exhibited gross hemorrhage (usually in the gut), sustained intracranial hemorrhages and fractured skulls, or died within 20 minutes after removal from the drum. These were discarded from consideration. The remainder survived for at least one hour or two after removal from the drum.

Blood pressures were determined repeatedly on the same rat. The method, developed in this laboratory, is described by Duncan, Hyman and E. L. Chambers (3). It is based on the microscopic observation of arteriolar blood-flow in the interdigital web of the hind foot while the femoral artery is being occluded by a pneumatic cuff around the upper part of the thigh. The pressure of the normal, unanesthetized rat showed variations in different rats from 90 mm. to 140 mm. Hg, the pressure of the majority lying between 110 mm. and 120 mm. The accuracy of the method may be indicated by the fact that successive readings on the same rat by different observers were reproduced within differences of 2–4 mm. Hg. The readings were taken at the moment a flow was detected when the pressure was released on the femoral artery. They may be regarded as the systolic pressures. The advantage of the method is in the absence of any surgery in the procedure.

The hematocrit volumes were obtained by the Van Allen method and the plasma volumes by the dye method introduced by Keith, Rowntree and Geraghty.

The capillary circulation was observed simultaneously in the subcutaneous tissue of the skin between the toes of the hind leg and in the meso-appendix, a triangular flap of mesentery between cecum and ileum. In order to have the rat sufficiently anesthetized to permit exposure of the meso-appendix after the trauma, it was given a subcutaneous injection of sodium pentobarbital solution (0.45 cc. of 1 per cent solution per 100 grams body weight) immediately before placing it in the drum. The anesthetic then became effective at about the time the rat was removed from the drum. Since the trauma is one of direct injury to the viscera it was found necessary to offer some degree of protection to the region selected for observation in order to avoid local petechiae. This was accomplished by strapping the lower abdomen with a strip of 1 inch adhesive with cotton interposed directly over the cecum. The protection afforded was not sufficient to prevent the development of shock symptoms. The cecum of these rats was hyperemic but showed no bruises or local hemorrhages such as usually occur when the lower abdomen is unprotected. After exposing the cecum the meso-appendix was spread over a horse-shoe shaped ribbon of lucite. The viscus was covered with cotton and the preparation kept moist by a drip of Ringer Locke solution buffered to pH 7.4 and thermostatically controlled at 38°C.

Acknowledgments are made to Dr. George W. Duncan (Department of Surgery, The Johns Hopkins Hospital) who worked with us during the summer of 1941, to Chester Hyman and Edward L. Chambers who assisted in devising the blood pressure technique for the rat.

RESULTS. 1. *Evidence for the site of action of the trauma.* The rats in the

rotating drum suffered minor contusions over the bony prominences, principally the dorsal margin of the ilium, the scapula, the base of the skull, and the snout. The teeth were frequently broken, and the scrotal sac contused.

Experiments were made to determine which regions of the body had to be traumatized to induce fatal shock. The rats were protected over various regions with pads of absorbent cotton which were held in position with adhesive tape. It was found that padding over the head, shoulder, back and scrotum did not protect the animal provided the abdomen was left exposed. These rats went into fatal shock with the same number of revolutions as those left completely unprotected. When the abdomen was well-padded, the rat withstood 1000 revolutions without developing fatal shock.

In rats with the entire abdomen protected the blood pressure dropped to about 50 mm. Hg immediately after removal from the drum. In the great majority of cases the pressure then rapidly rose to normal values. Autopsies on these animals, killed at varying intervals after the trauma, revealed no gross visceral congestion.

The conclusion is that the action of the Noble-Collip drum, within the range of 650 revolutions, induces a fatal, shock-like state largely through direct trauma to the viscera.

2. *Signs and symptoms of the shocked state in unprotected rats. External signs.* These were the same as described by Noble and Collip (l. c.). Immediately on removal from the drum the rat was comatose. The ears, tail and feet showed an intense pallor and were cooler than normal. During the following 15–20 minutes the respiration which was at first shallow and rapid, became slower and more normal. The initial pallor decreased, and the rats became more responsive to cutaneous stimuli. This improvement persisted for a variable time, after which, in fatal cases, the animals became progressively less responsive until death.

Internal signs. The gastro-intestinal tract showed marked vascular engorgement chiefly in the duodenum, upper jejunum, cecum and, to a lesser extent, the lower ileum. Varying amounts of free fluid were usually present in the lumen of the gut, especially in the duodenum and upper jejunum. The mucosa frequently showed marked vascular engorgement, though no definite ulcerations were ever observed. The mesenteric lymphatics and the thoracic duct were occasionally engorged and, sometimes, the contained fluid was sanguinous. The liver, spleen, kidneys, and adrenals gave evidence of vascular engorgement, likewise the lungs which frequently contained petechiae. Gross examination of the brain revealed no pathology except in rats discarded because of cranial hemorrhage and fracture. The urine was scanty, concentrated, dark, and, in rare instances, grossly bloody.

Rectal temperature. There was a consistent drop in temperature from the normal of 98°–100° to 93°–95°F. immediately following drumming. It persisted below normal in all fatal cases.

Blood pressure. In some rats blood pressure readings were unobtainable because of complete circulatory stasis in the skin vessels. In about 250 cases only a few blood pressure readings were taken. In 50 cases readings were taken at 10 minute intervals on each rat over a period of 2–4 hours. Representative fatal

cases showed a primary fall to 30–40 mm. Hg, followed, within 10–20 minutes, by a temporary rise to 60–70 mm. Hg; after which the pressure gradually fell during a period of 2–4 hours until death ensued. A number of cases in this group maintained extremely low pressures with no rise throughout until death.

Blood pressure curves of the rats which survived also showed an initial drop which persisted for about 10–20 minutes and then rose to the normal level within 1 to 20 hours.

Hematocrit. Blood samples were withdrawn from the jugular vein at 15–20 minute intervals after removal from the drum. Because of the small total blood volume of the rat, care was taken to remove minimal amounts, on the average, not over 0.3–0.5 cc. Hematocrit volumes were determined in duplicate in about 50 rats using the Van Allen method.

Hemoconcentration was consistently obtained. In those which recovered, the hematocrit values never rose more than 6–8 per cent above normal, and normal levels were reached within 1–3 hours after the trauma. In those which eventually died (1–12 hours after the trauma) the hematocrit values began to rise 30–60 minutes after removal from the drum. Levels of 9–20 per cent above normal, with an average of 12 per cent, were reached within 1–3 hours, which persisted until death. These results are in accord with the hemoglobin determinations reported by Noble and Collip (l. c.).

Plasma volumes. Plasma volumes were determined by the T-1824 dye method. The procedure employed was essentially that described by Beckwith and Chanutin (4). The dye determinations were taken on samples from the right atrium. Experience of other investigators with shocked dogs has indicated that samples from veins gave variable results.

All the rats were subjected to drum trauma within 24 hours after the control determination. In all the cases in which there was no gross evidence of hemorrhage or fluid loss into the gut, the trauma was found to induce a decrease in total plasma volume of about 12 to 15 per cent. A maximum value of 20 per cent was obtained in cases in which gross hemorrhage was visible in the duodenum. Parallel hematocrit readings were not done.

3. *Diffusion of the dye, T-1824, from blood vessels.* An indication of the site of fluid loss was obtained by observing the escape of the dye, T-1824, intravenously injected prior to trauma. Gross examination showed that the skeletal muscles were diffusely bluer than in non-traumatized rats. There was also evidence of considerable loss of the dye into the gastro-intestinal tract, over bony prominences and, in females, into the subserosal regions of the uterus and its cornu.

The change affected by the drum trauma to diffusion of the dye T-1824 was also tested by the local application of heat before and after the trauma. Tests were first made on normal rats by injecting T-1824 (0.2 to 0.3 cc. of a 1 per cent solution in mammalian Ringer) into the jugular vein. Three regions of the skin on one side of the abdomen were then exposed to heat for 10, 20 and 30 seconds, respectively, by applying glass tubes through which hot water was flowing, the temperature at the outflow being kept at 46°C. No bluing effect was noted in any of the heated regions. The same rat was then subjected to 600

revolutions. Upon removal from the drum, the regions of the abdomen previously exposed to heat still showed no color change. The other side of the abdomen was then treated with the same heat-application technique. The region exposed for 20 seconds was slightly blue while that exposed for 30 seconds was a deeper blue.

4. *Visceral and cutaneous capillary circulation.* The following data represent observations on tissues in which the effects of direct trauma tended to be superimposed on the more generalized shock condition. The description of the observed changes in the capillary circulation was taken from 20 rats.

a. The effect of the trauma on the circulation was studied by exposing the meso-appendix of rats at varying intervals after they had been drummed for 650 revolutions. Two of the criteria, that were especially noted, were the sensitivity of the muscular vessels of the capillary bed to epinephrine, and their vasomotion. This vasomotion is independent of the arterial pulsations and consists of a widening and a narrowing in slow, irregularly periodic sequences, varying from about 15 seconds to 3 minutes (5).

Five minutes after removal from the drum the capillary circulation was hyperemic and slow. The terminal arterioles were partially dilated and lacked the vasomotion characteristic of arterioles in normal rats. However, there was a pulsatile flow evidently transferred from the arteries. When first observed, the circulation in the larger vessels was slow but it gradually became accelerated, a condition which was found to correspond with the appearance of external signs of improvement noted in unanesthetized rats. In some cases a reversal of flow occurred in the smaller veins, the blood backing into the capillaries. The veins and arteries were hypersensitive to epinephrine. A concentration of 1 part in 2 million, which in the control, nembutalized rat induces slight arteriolar constriction, completely occluded the terminal arterioles and induced contractions of the veins followed by uneven relaxations and irregular constrictions which lasted 20–30 minutes.

Thirty minutes after removal from the drum, hemoconcentration was indicated by the closer packing than normally of the blood corpuscles in the capillaries while the flow was appreciably slowed in the arteries and veins and sluggish in the capillaries. The indications were that the hemoconcentration either had occurred previous to the period of observation or was occurring remote from the area under observation. The terminal arterioles and precapillaries still showed no vasomotion and now were hyposensitive to epinephrine, constricting only with concentrations of 1 part in 1 million or higher.

The application of heat, by causing the drip of Ringer's solution to rise to 40°C, resulted in the development of hemorrhages throughout the bed, indicating an abnormal fragility of the capillary walls. In the normal rat this amount of heat produced only a hyperemic flow with no sign of local hemorrhage.

Sixty or more minutes after removal from the drum the capillary bed was relatively ischemic with very little flow. Most of the blood coming from the arteries by-passed the capillary bed by flowing directly through cross shunts to the corresponding veins. The arteries were considerably constricted. The smaller venules contained a sluggish and resurgent flow with a tendency of the

blood cells to be stagnated. Considerable back-flow from the veins into the capillary bed was observed, causing further stagnation.

About 10–15 minutes before death, a sticking of leucocytes became evident in the walls of the smaller veins and venules. Responses of the muscular vessels to epinephrine were extremely poor, the application of a concentration of 1 part in 1 million yielding little or no response.

b. Peripheral circulation in the interdigital web and scrotum. Immediately on removal from the drum the blood in the capillary beds was found to be stagnant, the blood cells in the capillaries merely oscillating to and fro. There was no evidence, however, of true stasis in which the red cells become closely packed through the loss of plasma from the vessel. In some of the more deeply placed arterioles a slow, continuous flow usually persisted until shortly before death. The superficial venules were narrowed and appeared abnormally pale, containing only a very few red cells. No recovery of flow occurred in those rats which died within 2–3 hours after the trauma.

SUMMARY

1. *Blood pressure.* Exposure of 150 gram rats to 600–650 revolutions in the Noble-Collip drum resulted in a lowered blood pressure which, in fatal cases, persisted below 60–70 mm.

2. *Hemoconcentration and plasma volume.* a. The hematocrit values always showed an increase, averaging 12–15 per cent above the initial normal values. b. The total plasma volume in the majority of cases, showed a decrease of about 12–15 per cent.

3. *Site of trauma.* Protection of the abdomen during the drumming prevented the development of a shock-like state in rats subjected to drumming up to 1000 revolutions.

4. *Capillary circulation in the rats subjected to 650 revolutions of the drum.* a. *Visceral.* i. The hyperemic state of the capillary circulation in the mesentery was probably due, in large part, to the direct trauma on the viscera. A slowing of the flow and absence of arteriolar vasomotion progressively became apparent concurrently with the fall in blood pressure. ii. The sensitivity to epinephrine was at first increased, particularly of the veins. It then progressively decreased to considerably below the normal response. iii. Hemoconcentration became visibly apparent within 30 minutes after the trauma. iv. In the terminal stages, the flow became increasingly restricted and was accompanied by a progressive stagnation, especially in the venous portion of the capillary bed. b. *Peripheral.* From the time of removal from the drum until death, the circulation was found to be stagnant, with a slow flow only in a relatively few vessels. There was no sign of local hemoconcentration.

REFERENCES

(1) NOBLE, R. L. AND J. B. COLLIP. Quart J. Exper. Physiol. **31**: 187, 1942.
(2) NOBLE, R. L. AND J. B. COLLIP. Quart. J. Exper. Physiol. **31**: 201, 1942.
(3) DUNCAN, G. W., C. HYMAN AND E. L. CHAMBERS. J. Clin. Med. (in press).
(4) BECKWITH, J. R. AND A. CHANUTIN. Proc. Soc. Exper. Biol. and Med. **46**: 66, 1941.
(5) CHAMBERS, R. AND B. W. ZWEIFACH. (In press.)

PLASMA COAGULATION AND FIBRINOGENOLYSIS BY PROSTATIC FLUID AND TRYPSIN[1]

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Both crude and crystalline trypsin are known to clot oxalated blood (1–3). Eagle and Harris (3) discovered that trypsin does not clot fibrinogen and that in appropriate amounts trypsin digests fibrinogen and fibrin, and clots oxalated plasma; trypsin also digests thrombin, and prothrombin (4). Ferguson and Erickson (5) found that crystalline trypsin, 1–2 mgm. acting alone were optimal for clotting citrated plasma, but when potentiated by calcium ions smaller amounts yielded quick solid clots.

Huggins and Neal (6) observed that the prostatic secretion of man and dog normally contains proteolytic agents capable of digesting fibrinogen and fibrin of certain species. The agent which disolves fibrin closely resembles the fibrinolysin obtained as a metabolic product of hemolytic streptococci by Tillett and Garner (7). Evidence was presented that this prostatic fibrinolysin was different from the activity which destroyed fibrinogen, fibrinogenase. Both proteolytic agents are present in human and dog prostatic fluids but in different amounts; human prostatic secretion contains much more fibrinolysin than fibrinogenase and canine prostatic fluid the reverse. Moreover, trypsin as recognized by the splitting of denatured hemoglobin according to the method of Anson (8) is constantly present in dog prostatic fluid and less often in human secretion.

In this paper we report the coagulation of oxalated plasma by prostatic fluid of the dog (DPF) and compare the clotting and fibrinolytic properties of prostatic fluid and trypsin.

METHODS AND MATERIALS. Prostatic fluid from 15 dogs was obtained by pilocarpine stimulation following the prostatic isolation procedure of Huggins, Masina, Eichelberger and Wharton (9); this fluid was sterilized by passing it through a Seitz filter. Human prostatic fluid from eight normal men was procured by massage of the prostate, *per rectum*, and was tested immediately after centrifugation.

Oxalated plasma was used throughout. Potassium oxalate, 2–8 mgm. per 1 cc. of blood was used as anticoagulant. Rabbit blood was obtained by cardiac puncture in a syringe containing the oxalate dissolved in saline. Certain specified samples were recalcified with calcium chloride, 1.5 per cent, 0.33 cc. The commercial trypsin used, "Trypsin 1:250", was manufactured by the Difco Laboratories. It was dissolved in saline and filtered; the pH of the solution, determined electrometrically, was 6.5. Heparin employed was that of Hynson, Westcott and Dunning and was freshly dissolved in saline before using; the manu-

facturers stated that 1 mgm. of this preparation prevented the coagulation of 7.5 cc. of cat's blood for 24 hours. Trypsin content of prostatic fluid was determined by the method of Anson (8) and the results are expressed in the units of Kolm, Shay and Gershon-Cohen (10).

Beef plasma was freed of prothrombin by adsorption with magnesium hydroxide and fibrinogen was prepared by the method of Smith, Warner and Brinkhous (11).

In all of the experiments, plasma 1 cc., was mixed with 0.5 cc. of fluid to be tested, either undiluted or diluted with saline and immediately placed in a water bath at 37°C.; the times of coagulation and of lysis were determined. Unless otherwise stated, *all of the data in this paper refer to the effect on plasma, 1 cc.*

RESULTS. *Coagulant action of prostatic fluid.* DPF (from 15 dogs) always induced firm coagulation of oxalated rabbit plasma without added calcium. The calcium content of DPF is low, 0.3 mM per kgm. of water (9). The time of clotting induced by DPF varied from 4 to 31 minutes, the usual time being about 10 minutes. With increasing dilution of DPF the clotting time became progressively increased. In a typical experiment 1 cc. of oxalated plasma was clotted by 0.5 cc. of DPF in 9 minutes; by 0.1 cc. in 20 minutes; by 0.05 cc. in 35 minutes; and by 0.006 cc. in 5 hours. With 9 specimens of oxalated beef plasma, DPF induced clotting in 22–50 minutes, without added calcium. In two instances diluted DPF, 0.006–0.05 cc. induced clotting of dog plasma, while in stronger concentrations no clotting occurred and the fibrinogen was destroyed. DPF never induced clotting of human blood in any concentration in 12 tests.

The prostatic fluid of 8 men always failed to clot oxalated rabbit plasma. The addition of calcium chloride after 4–21 hours resulted in a prompt, firm clot.

Effect of excess oxalate and of heparin on coagulation of rabbit plasma by DPF. In drawing rabbit blood, precautions were taken to minimize spontaneous thrombin formation. In addition to drawing the blood in a syringe containing liquid oxalate, the first 10 cc. of blood flowing from the heart were discarded and with the needle still in place a second sample was drawn into a fresh syringe. DPF consistently induced clotting of this oxalated plasma. An excess of potassium oxalate, 16 mgm. per 1 cc. of blood, still permitted this firm coagulation.

The addition of heparin, 3 mgm. to 10 mgm. per 1 cc. of blood modified the coagulation of oxalated rabbit plasma by DPF in two respects. Instead of a firm clot, a heavy flocculation occurred in the plasma, which remained liquid. On microscopic examination, the coagulum consisted of interlaced fibrils resembling fibrin. No precipitation occurred in the plasma, from which the clot had been removed by centrifugation, on heating to 56° or on half saturation with NaCl. This flocculation occurred at a slower rate than did the coagulation of plasma by DPF without heparin; the flocculation developed in 21 minutes to 2 hours, as compared with its coagulation in ten minutes without heparin.

Effect of heating on dialysis of DPF. Storing DPF in a refrigerator at 4°C. did not result in a loss of thrombin or fibrinolytic activity. The dialysis of DPF in a cellulose bag against running tap water at 4°C. for 5 days had no effect on its ability to clot oxalated dog or rabbit plasma or to effect subsequent fibrinolysis.

Ashing DPF at 550°C. with resolution of the ash in distilled water equal to the original amount abolished its coagulant and lytic actions.

Heating DPF at 60°C. for 30 minutes still permitted it to coagulate rabbit plasma in the same time as occurred with unheated DPF; heating to 70°C. for 10 minutes abolished the clotting action of DPF. Exactly similar findings were observed with heated trypsin solutions in saline.

Coagulative capacity of DPF on prothrombin and fibrinogen. DPF always failed to clot purified beef fibrinogen solutions. When prothrombin was added to a mixture of DPF and fibrinogen clotting occurred in 6 minutes. Clotting was not observed when DPF was added to beef plasma from which prothrombin had been adsorbed with colloidal $Mg(OH)_2$ after adjusting the adsorbed plasma

TABLE 1

The effect of dog prostatic fluid on coagulation and on lysis of fibrinogen and fibrin of plasma

One cubic centimeter of oxalated plasma + 0.5 cc. of prostatic fluid, undiluted or diluted to 0.5 cc. In series A, $CaCl_2$ was added immediately after mixing; in series B, $CaCl_2$ was added 6 hours after incubation.

	AMOUNT OF PROSTATIC SECRETION IN TEST (CC.)							
	0.5	0.1	0.05	0.025	0.016	0.01	0.006	0.005
Human plasma: Series A, calcium added immediately								
Coagulation (minutes)...	none	4	4	4	4	4	4	4
Fibrinolysis (hours).....		7	18	none	none	none	none	none
Human plasma: Series B, no added calcium								
Coagulation (minutes)...	none	none	none	none	none	none	30*	30*
Fibrinolysis (hours)......							none	none
Rabbit plasma: no added calcium								
Coagulation (minutes)...	10	30	30	180	180	5*	5*	5*
Fibrinolysis (hours)......	16	16	16	none	none	none	none	none

* No clotting in 6 hours; calcium then added.

to pH 7.4. DPF added to phosphated plasma produced clotting in 20 minutes. It was assumed that the magnesium solution had more effectively removed prothrombin than did calcium phosphate.

Trypsin content of DPF. The tryptic activity of 12 fresh specimens of DPF on denatured hemoglobin was determined (8) and values were obtained ranging between 0.1 to 1.1 unit, an average of 0.59 unit; 1 trypsin unit represents such tryptic activity as develops chromogen equivalent to tyrosine, 1 mgm., in 1 minute under the stated conditions, expressed per 100 cc. (10).

Action of prostatic fluid and trypsin on human and rabbit plasma. DPF added to oxalated human plasma never induced coagulation but destroyed fibrinogen in concentrations as low as 0.01 cc. (table 1). Prompt clotting occurred in the unclotted specimens when beef fibrinogen was added after incubation for 6

hours. Added to oxalated rabbit plasma, coagulation occurred with as little DPF as 0.016 cc.

Trypsin in 10 mgm. amounts destroyed human and rabbit plasma fibrinogen before clotting occurred. Trypsin in amounts 1 mgm. to 5 mgm. caused clotting of human plasma, but smaller quantities were ineffective (table 2). Rabbit plasma was clotted with much smaller amounts of trypsin than human plasma; trypsin in amounts of 0.1 to 5 mgm. being effective. Human fibrin was digested with slightly less amount of trypsin than rabbit fibrin.

Heparin was found to interfere with the digestive action of trypsin on fibrinogen. In the presence of heparin 3 mgm., trypsin 10 mgm. produced flocculation of dog plasma, an amount which promptly destroyed fibrinogen in the absence of heparin.

TABLE 2

Coagulation and fibrinolysis induced by trypsin in human and rabbit plasmas
One cubic centimeter of oxalated plasma + 0.5 cc. trypsin solution

	AMOUNT OF PANCREATIC TRYPSIN IN TEST (MGM.)								
	10	5	3	2.5	2.0	1.5	1.0	0.5	0.1
Human plasma									
Coagulation (minutes)....	none	15	15	15	15	40	18 hr.	none	none
Fibrinolysis (hours).......		$\frac{1}{4}$	5	18	18	none	none	none	none
Rabbit plasma									
Coagulation (minutes)....	none	3	15	15	15	15	15	90	18 hr.
Fibrinolysis (hours).......		$\frac{1}{4}$	5	18	none	none	none	none	none

DISCUSSION. Pancreatic trypsin according to its concentration affects plasma in various ways. Large amounts destroy fibrinogen, smaller quantities coagulate oxalated plasma and subsequently the fibrin dissolves, while still smaller amounts clot plasma without destroying fibrin (3). Ferguson (15) has concluded that both crude trypsin and pure crystalline trypsin show parallelism of behavior with respect to fibrinogenolysis and fibrinolysis.

Human prostatic secretion has a powerful fibrinolytic action but destroys fibrinogen at a slow rate (6). This fluid never clots oxalated rabbit plasma and *inconstantly contains trypsin according to the method of Anson*. This is further evidence that the principal proteolytic enzyme of human prostatic fluid is fibrinolysin similar to the fibrinolysin obtained from hemolytic streptococci by Tillett and Garner (7) and that it differs in its activity from the main proteolytic enzyme of prostatic fluid of the dog and from pancreatic trypsin.

Many of the properties of DPF resemble trypsin, which is constantly detectable in small amounts in this fluid. Both DPF and trypsin coagulate oxalated rabbit plasma but not fibrinogen or plasma from which prothrombin has been adsorbed. Both clot rabbit plasma in concentrations too low to cause fibrinolysis. Both the commercial trypsin used and DPF were active after heating at 60°C. for 30 minutes and both were destroyed at 70°C. Heparin added to oxalated rabbit plasma did not prevent coagulation by trypsin or DPF.

Ferguson and Glazko (12) found that heparin decreased the proteolytic activity of trypsin and in line with this observation we found that heparin, 3 mgm. permitted trypsin 10 mgm. to clot oxalated plasma, an amount which destroyed fibrinogen before clotting in the absence of heparin.

There are also points of similarity between the proteolytic activity of DPF, trypsin and the enzyme prepared from chloroform treated plasma by Tagnon and associates (13, 14). Tagnon's preparation from dog plasma (13) clotted plasma containing prothrombin, but did not coagulate fibrinogen. At low concentration, the chloroform plasma globulin from human blood (14) clotted fibrinogen while at higher concentration coagulation was followed by fibrinolysis and in still larger amounts fibrinogenolysis resulted with no coagulation.

One important difference was observed between the proteolytic effects of trypsin and the chloroform plasma globulin on the one hand and DPF, in that DPF has far greater activity in destroying human plasma fibrinogen than in destroying fibrin; with diluted amounts of DPF, fibrinogen is digested before clotting occurs while the same amounts did not cause fibrinolysis of blood which had been recalcified. Trypsin in large amounts destroys fibrinogen promptly while smaller amounts are ineffective. The smallest amounts of DPF clotting rabbit plasma destroyed human fibrinogen in 5 hours while the smallest amounts of trypsin effecting clotting did not digest this protein (tables 1, 2).

It should be stressed that the enzymes of prostatic fluid have not been isolated in a chemically pure state and that deductions at the present time are based on the physiological activity of these fluids. Much evidence has shown the proteases are to be considered as "enzyme and inhibitor" systems: until the conditions and degree of activation of these modalities are more clearly defined than at present conclusions drawn as to the chemical nature of the prostatic proteases must be tentative. However, it is concluded that the proteolytic properties of DPF are functionally not identical with pancreatic trypsin, although the similarity of many properties suggests that fibrinogenase and trypsin are closely related.

SUMMARY

The prostatic secretion of normal dogs in effective amounts constantly clots oxalated rabbit and beef plasmas, and a mixture of fibrinogen and prothrombin, but does not coagulate fibrinogen alone or prothrombin-free plasma. This clot formation proceeds in the presence of large amounts of oxalate and heparin, although with heparin a flocculated precipitate develops instead of a firm clot. Dog prostatic fluid does not coagulate human plasma since fibrinogen is destroyed before clotting occurs. Thrombic activity of dog prostatic fluid is stable for months at 4°C. It does not disappear on prolonged dialysis against tap water or on heating at 60°C. for thirty minutes but is destroyed at 70°C.

Human prostatic fluid does not coagulate oxalated plasma.

Many proteolytic properties of dog prostatic fluid resemble those of pancreatic trypsin; an important difference is the greater activity of prostatic secretion even

in low dilution in destroying plasma fibrinogen compared with fibrin. Trypsin does not possess this effect.

The principal proteolytic activity of dog prostatic fluid, *fibrinogenase*, resembles, but is not physiologically identical with, trypsin; the chief proteolytic enzyme of human prostatic fluid is *fibrinolysin*.

REFERENCES

(1) HEARD, W. N.   J. Physiol. **51**: 294, 1917.
(2) NORTHROP, J. H. AND M. KUNITZ.   J. Gen. Physiol. **16**: 295, 313, 1932.
(3) EAGLE, H. AND T. N. HARRIS.   J. Gen. Physiol. **20**: 543, 1936.
(4) FERGUSON, J. H.   Proc. Soc. Exper. Biol. and Med. **46**: 80, 1941.
(5) FERGUSON, J. H. AND B. N. ERICKSON.   This Journal **126**: 661, 1939.
(6) HUGGINS, C. AND W. NEAL.   J. Exper. Med. **76**: 527, 1942.
(7) TILLETT, W. S. AND R. L. GARNER.   J. Exper. Med. **58**: 485, 1933.
(8) ANSON, M. L.   J. Gen. Physiol. **22**: 79, 1938.
(9) HUGGINS, C., M. H. MASINA, L. EICHELBERGER AND J. D. WHARTON.   J. Exper. Med. **70**: 543, 1939.
(10) KOLM, R., H. SHAY AND J. GERSHON-COHEN.   J. Lab. and Clin. Med. **27**: 835, 1942.
(11) SMITH, H. P., E. D. WARNER AND K. M. BRINKHOUS.   J. Exper. Med. **66**: 801, 1937.
(12) GLAZKO, A. J. AND J. H. FERGUSON.   Proc. Soc. Exper. Biol. and Med. **45**: 43, 1940.
(13) TAGNON, H. J.   Science **95**: 334, 1942.
(14) TAGNON, H. J., C. S. DAVIDSON AND F. H. L. TAYLOR.   J. Clin. Investigation **21**: 525, 1942.
(15) FERGUSON, J. H.   J. Gen. Physiol. **25**: 607, 1942.

COPPER-INDUCED PSEUDOPREGNANCY FACILITATED BY PRETREATMENT WITH ESTROGEN[1]

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In a previous report (1) it was shown that pseudopregnancy can be induced in the rat by an intravenous injection of a copper solution at the time of estrus. Furthermore it was shown that the estrous rhythm of the rat is not appreciably altered by similar injections during the metestrous or diestrous phases of the cycle. These results suggested the possibility that the responsiveness of the rat varies with the cyclic fluctuations of endogenous estrogen. If this is the case, pretreatment with estrogen should make it possible to induce pseudopregnancy by injections of copper solution at other stages of the estrous cycle. The experiments described in this paper were designed to test this possibility.

MATERIALS AND METHODS. The rats used in this study were of the Wistar strain, bred and raised in our stock colony. Six to 8 rats were kept in a cage and the experimental observations were started after they were 80 to 150 days of age. Individual daily vaginal smear records were kept throughout the investigation until the animals were sacrificed. As a rule the smears were taken at about 9 o'clock a.m. each day and the rats were used only after they had exhibited two or more normal estrous cycles. After an injection, a prolonged diestrus (10 to 14 days) was considered evidence of an induced pseudopregnancy. The technique of the intravenous injection and the preparation of the copper acetate solution was the same as described previously (1).

The estrogen used in these experiments was crystalline estrone[3] dissolved in sesame oil. In order to avoid the toxic effects as described by Crafts (2) the oil was treated by the method of Bruce and Tobin (3) and the dose kept very small. In our experience, volumes of oil greater than 0.1 cc. are not absorbed from subcutaneous sites within a 24 hour period. By the use of a special 0.25 cc. Luer syringe it was possible to inject doses ranging from 0.01 cc. to 0.05 cc. in volume. The concentration of the estrone in the oil was 400 gamma per cubic centimeter.

EXPERIMENTAL. Fifty rats were given an intravenous injection of 1 per cent copper acetate solution (0.1 cc.) at the time of estrus with a resultant pseudopregnancy in every case. Seventeen rats were injected with copper at the time of metestrus and 17 at the time of diestrus but pseudopregnancy did not occur in any case. However, when estrone (8 to 12 gamma) was given during the

afternoon of the day of an estrous smear and then the copper was given the next morning (normally metestrus) there was a resulting pseudopregnancy in every case (37 rats shown in table 1). Similarly when estrone (8 to 12 gamma) was given on the day of metestrus and then the copper given on the next day (normally diestrus) pseudopregnancy was induced in a majority of the rats.

Varying the dose of estrone makes it apparent that a dose of 4 gamma is effective when given at the time of estrus but not when given at metestrus. Similarly, doses of 8 to 12 gamma of estrone are not uniformly effective when given at metestrus. The doses of estrone used in this study did not appreciably alter the rhythm of the vaginal smears except that estrone given at estrus

TABLE 1

Summary of results showing how estrogen pretreatment facilitates the action of copper acetate in adult female rats

NUMBER OF RATS USED	PHASE OF CYCLE AND TREATMENT			PSEUDOPREGNANCY INDUCED		
	Estrus	Metestrus	Diestrus	No	Yes	Per cent yes
50	Cu			0	50	100
17		Cu		17	0	0
17			Cu	17	0	0
5	Sesame oil			5	0	0
17	Sesame oil	Cu		17	0	0
31	Estrone 4 to 12γ			31	0	0
7	Estrone 4γ	Cu		2	5	71
37	Estrone 8 to 12γ	Cu		0	37	100
6		Estrone 4 to 12γ		6	0	0
4		Estrone 4γ	Cu	4	0	0
8		Estrone 8 to 12γ	Cu	2	6	75
3		Estrone 16 to 24γ	Cu	0	3	100
7	Estrone 30 to 100γ			0	7	100

Cu denotes an intravenous injection of 0.1 cc. of a 1 per cent solution of copper acetate.
Sesame oil, subcutaneous injections ranging from 0.03 to 0.05 cc.
Estrone in sesame oil 400 gamma per cc. given subcutaneously.
Since these three phases of the estrus cycle come on successive days the interval between estrone and the copper injections was 18 to 20 hours.

maintains the cornification stage for the next day or two. These results indicate that an appropriate dose of estrogen makes the rat as responsive to an injection of copper solution at other stages of the cycle as it normally is at estrus.

A note of caution should be added here since it has been found that larger doses of estrone (30 gamma or over) given at the time of estrus will induce pseudopregnancy. In other experiments (not shown) single injections of 40 gamma given at estrus have caused the same cessation of cycles as was produced by the prolonged daily injections of 40 R. U. of estrone reported by Merckel and Nelson (4). Astwood and Greep (5) had reported that toxic materials would induce pseudopregnancy so a number of controls were given sesame oil

with and without subsequent injections of copper but the tests were uniformly negative as shown in table 1.

Since the completion of these observations on the rat the study has been extended by one of us (J. T. B.) to include the rabbit. Copper injected intravenously into the estrous rabbit induces ovulation and pseudopregnancy (6). However a series of tests have shown that anestrous rabbits do not ovulate after an intravenous injection of copper solution. Table 2 summarizes the results

TABLE 2

Summary of results showing that estrogen pretreatment of the anestrous rabbit makes it possible to induce ovulation by copper acetate

RABBIT	R ESTROGEN		R COPPER	OVULATION RESPONSE
	Date	Date	Date	
1			9/1	Negative
2			9/1	Negative
3			9/1	Negative
4			9/1	Negative
5			8/24	Negative
5	10/20	10/21	10/22	Positive
6			9/1	Negative
6	10/20	10/21	10/22	Positive
6			11/23	Negative
6	12/8	12/9	12/10	Positive
7	10/20	10/21	10/22	Positive
7			11/23	Negative
7	12/7	12/8	12/9	Positive
8	12/29	12/30		Negative
9	12/29	12/30		Negative
10	12/29	12/30		Negative
11	12/29	12/30		Negative
Controls (estrous rabbits)				
12			8/24	Positive
13			10/22	Positive
14			11/23	Positive
15			11/23	Positive
16	12/16	12/17		Negative
17	12/16	12/17		Negative

Dosage: Estrogen, 10,000 I.U. subcutaneously. Copper, 1 cc. of a 1 per cent solution of copper acetate intravenously.

obtained when anestrous rabbits were given an injection of 1 cc. of a 1 per cent solution of copper acetate. Ovulation was induced in all 4 control rabbits but in none of the 7 anestrous rabbits. However when the anestrous rabbits were given 10,000 I. U. of estrogen[4] subcutaneously on each of two successive days and then were given copper solution on the third day, ovulation occurred in each case (5 times in 3 rabbits). These rabbits were considered anestric on the

[4] This estrogen (40,000 I.U. per cc. in propylene glycol) was generously supplied by Dr. J. P. Schooley, Difco Laboratories Inc.

basis of having a tiny atrophic uterus judged by abdominal palpation and verified at laparotomy.

DISCUSSION.   The apparent enhancement of the sensitivity of the adult rat and the anestrous rabbit by pretreatment with estrogen may be an effect similar to that reported by Lane (7).   He found that small doses of estrogen given for a short period of time seemed to stimulate the ovaries of the immature rat. Fevold et al. (8) and Frank and Berman (9) found that pretreatment with estrogen augments the ovarian response to gonadotropic extracts.   Cytological studies of the pituitary indicate that the initial effects of estrogen may bring about a discharge of gonadotropic hormone (10).

There is also the possibility that estrogen pretreatment exerts its effect at least in part through the nervous system since the work of Brooks et al. (6) indicates that copper induces ovulation by an excitation of the central nervous system.   Boling and Blandau (11) have shown that appropriate doses of estrogen and progesterone will induce a state of sexual receptivity in the guinea pig and rat.   Ball (12) studied the effect of estrogen on sex behavior and concluded, "that the gonadal hormone does not organize the mating behavior pattern in the adult but merely activates a pattern already present."   It seems possible therefore that estrogen may sensitize certain neural mechanisms which normally function only at estrus.   In our experiments it seems that copper is an effective stimulus only when some mechanism (possibly neural) has been activated by estrogen.

SUMMARY

1. Intravenous injections of 0.1 cc. of a 1 per cent copper acetate solution induce pseudopregnancy in rats if injected at the time of estrus.   This dose of copper does not induce pseudopregnancy if injected during metestrus or diestrus.

2. Pretreatment with estrogen makes it possible to induce pseudopregnancy by injection of copper during metestrus or diestrus.

3. The dose range of estrogen which makes metestrous and diestrous rats equally as susceptible as the normal estrous rat to the stimulus of copper has been determined.

4. Pretreatment with estrogen makes it possible to induce ovulation and pseudopregnancy in the anestrous rabbit by an intravenous injection of copper acetate.

REFERENCES

(1) DURY, A. AND J. T. BRADBURY.   This Journal 135: 587, 1942.
(2) CRAFTS, R. C.   Endocrinology 31: 142, 1942.
(3) BRUCE, R. A. AND C. E. TOBIN.   Endocrinology 27: 956, 1940.
(4) MERCKEL, C. AND W. O. NELSON.   Anat. Rec. 76: 391, 1940.
(5) ASTWOOD, E. B. AND R. O. GREEP.   Proc. Soc. Exper. Biol. and Med. 38: 713, 1938.
(6) BROOKS, C. M., W. G. BEADENKOPF AND S. BOJAR.   Endocrinology 27: 878, 1940.
(7) LANE, C. E.   This Journal 110: 681, 1935.
(8) FEVOLD, H. L., F. L. HISAW AND R. O. GREEP.   This Journal 114: 508, 1936.
(9) FRANK, R. T. AND R. L. BERMAN.   Endocrinology 28: 211, 1941.
(10) SEVERINGHAUS, A. E.   Sex and internal secretions.   The Williams & Wilkins Co., 1939.
(11) BOLING, J. L. AND R. J. BLANDAU.   Endocrinology 25: 359, 1939.
(12) BALL, J.   J. Comp. Psychol. 24: 135, 1937.

THE EFFECT OF EXPERIMENTAL THYROID ABNORMALITIES ON APPETITE

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Our purpose in this investigation was to determine the composition of a diet which rats would choose if they could exercise choice, similarly as do people. After this dietary composition was determined by weighing the foods eaten by the rats, we then wished to make some animals hypothyroid by thyroidectomies, and others hyperthyroid by feeding them desiccated thyroid tissue. A recent clinical study on hyperthyroid patients (8) indicated uniformly low protein intake, as compared with a group of patients with supposedly normal thyroid activity.

There is an extensive literature which deals with the free-choice, or self-selection, or cafeteria-style method of feeding animals and children which we shall not review here (see references 1–7). Earlier studies have shown that within certain limitations, food selection as guided by appetite is a good method of promoting growth and health in animals and infants (7). Certain deficiency diseases have been found to be compensated for by modifications in dietary selection (6), but this is not always the case.

METHODS. 1. *Food selection.* We kept several groups of newly weaned male rats in individual round wire cages. Each rat was kept in an individual round wire cage, surrounded by six food cups. These cups contained casein, dextrose, leaf lard, baker's yeast (grown on molasses and killed), powdered agar, and Osborne and Mendel's salt mixture. We added a daily cod-liver oil supplement of one drop (1000 U.S.P. units of vitamin A and 100 U.S.P. units of vitamin D). Agar was added to supply bulk and is not further considered in this paper.

The food cups were weighed every four days, and filled again to a predetermined weight in grams. We used 100 cc. glass beakers as food cups, and avoided spillage by a hollow metal cylinder insert. In this cylinder we soldered a downward-sloping shelf, with a $\frac{3}{4}$-inch hole in the middle.

2. *Basal metabolic rates (BMR's).* The apparatus used was of the type which measures oxygen consumption. The animal container was a large glass desiccator jar, in which the animal sat on a wide-mesh wire screen. The bottom of the jar contained saturated salt water, which deodorized excreta and kept the humidity relatively low. The animal received oxygen from a rectangular float which moved a pointer on a large scale, so that the cubic centimeters of oxygen used could be read off. Soda lime was placed in a wire "collar" surrounding the animal for the absorption of carbon dioxide. During the routine tests, the carbon dioxide concentration in the animal chamber was about 0.10 per cent, and the oxygen concentration was about 21.5 per cent. Three such entire

apparatus were set in soft rubber and housed in a noiseless constant temperature chamber. All readings could be made through a glass front, without opening the chamber. The rats were exposed to fairly bright lights during the tests, since this seemed to keep them more quiet.

Rats were fasted 18 hours prior to a BMR test. A test consisted of four measurements of oxygen consumption while the animal was quiet, each for a period of 15 minutes. The average of the two lowest readings was used as the basis for calculation. We calculated all our BMR's as calories per square meter of body surface per 24 hours, using the following formula:

$$\frac{24 \times cc. \ O_2 \ used/hr. \times 4.825 \times correct. \ T. \& P.}{9.1 \ \sqrt[3]{Wt. \ of \ animal^2}} = Cal./sq. \ m./24 \ hr.$$

The variation or "error" on repeated tests with the same animal was about 6 per cent by the above method.

3. *Thyroidectomies on rats.* A successful complete thyroidectomy was indicated by a notably coarser hair coat, by sluggishness, cessation of growth and by a drop in the BMR. Only about half of the thyroidectomies performed met these criteria over a period of months; the remainder showed an initial effect which wore off after a few weeks, and some of these animals later became very hyperactive.

4. *Hyperthyroid rats.* It was relatively easy to make animals hyperactive, with cessation of growth and a rise of 30 per cent to 50 per cent in the BMR. We mixed 100 mgm. of desiccated thyroid (Armour's) into a solution of 25 per cent cane sugar, and fed each rat a half cubic centimeter (i.e., 50 mgm. of thyroid) every two or three days, depending on how definitely hyperthyroid the animal became. Rats like sugar water, and licked this thyroid mixture directly off the tip of a tuberculin syringe.

RESULTS. 1. *General character of self-selection.* The survival of the species is proof that rats are able to find and select a diet in nature which supports growth and reproduction. The food elements found "in nature", however, are very likely to be mixtures of various constituents, and thus survival of the race does not answer the question whether the chemical detectors at the oral end of the organism are adequate to select an adequate diet from among chemically pure foods.

In a short preliminary experiment we fed 13 rats with four feeding cups in each cage, the cups containing casein, dextrin, lard, and a mixture of equal parts of yeast and salt mixture. After one month, 5 of these rats looked healthy and gained weight. The other 8 rats looked sick and lost weight. The intake of dextrin and lard in the two groups was not very different, but the casein intake was dramatically low for the sick group. This absence of protein appetite was possibly due to a low intake of yeast, as seen in table 1. This table presents the average intake per rat in grams per day of the two groups of rats.

We separated the salt mixture and the yeast into separate feeding cups, and in further experiments only about 1 rat in 20 failed to select a diet which supported growth. The character of self-selection in a typical normal rat over a

period of several months is illustrated in figure 1, and is discussed later. Figure 1 gives a curve of total caloric intake at the top; then three curves of caloric intake as protein, carbohydrate and fat. This rat was 3 months old at the beginning, and the dates give the time of year and duration of study. Below is a growth curve and the curve of normal BMR variation.

2. *Growth curves.* We charted the growth of individual rats, as well as of groups of rats, in order to have a constant objective criterion of the general welfare of our animals. The first indication of dietary insufficiency (because of poor selection or other reasons) was frequently a change of slope in the growth curve.

TABLE 1

Average food intake per rat per day

	CASEIN	DEXTRIN	LARD	SALTS AND YEAST	TOTAL
	grams	*grams*	*grams*	*grams*	*grams*
Healthy rats............	2.3	3.3	2.1	1.1	8.8
Sick rats...............	0.3	3.0	1.6	0.6	5.5
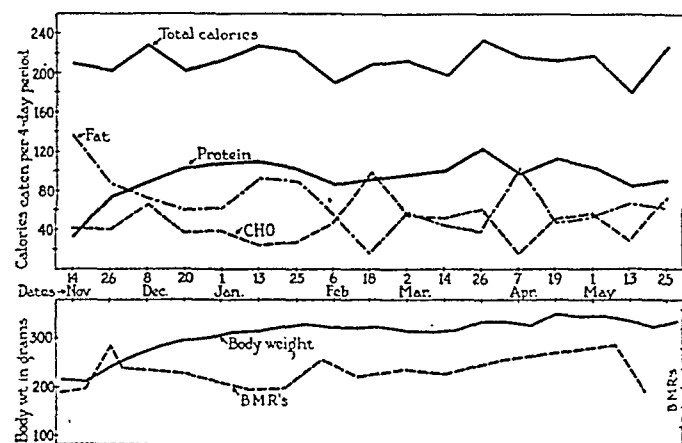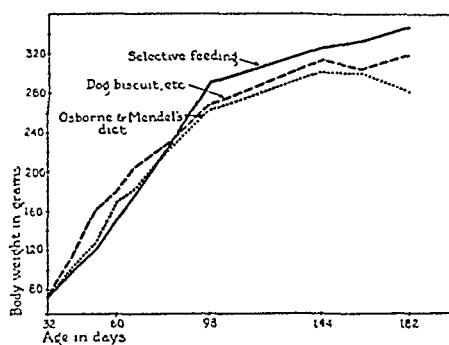
Fig. 1   Fig. 2

Fig. 2. Study of growth curves in 3 groups of litter-mate controls with 5 rats in each group

We found that the great majority of rats selected a diet which promoted normal growth. Figure 2 shows growth curves of 3 groups of litter-mate controls. One group received the standard Osborne and Mendel diet mixture, another was fed Purina dog biscuit and various other foods used for stock animals in our laboratory, and the third group was on selective feeding. During the first one and a half months the selecting rats did slightly less well than the others, but in the long run the selected diet seemed somewhat superior in promoting growth. The results of this experiment prompted us to use a larger number of animals over a longer period of time—an experiment that is still in progress.

Figure 3 shows the general trend of the individual growth curves of six normal

rats kept on selective feeding for almost a year, as well as growth curves of a typical hypothyroid and of a typical hyperthyroid rat.

3. *Age differences in food selection.*   In the course of our work we discovered that rats seem to undergo a change of appetite at the age of about 4 months. This is illustrated by the data in table 3.   The age of 4 months also marks a decrease in rate of growth (fig. 3).   In order to make possible comparisons among rats of various ages and sizes, all our data below was calculated in terms of grams or calories eaten *per 100 grams body weight* per 4-day period.   Then
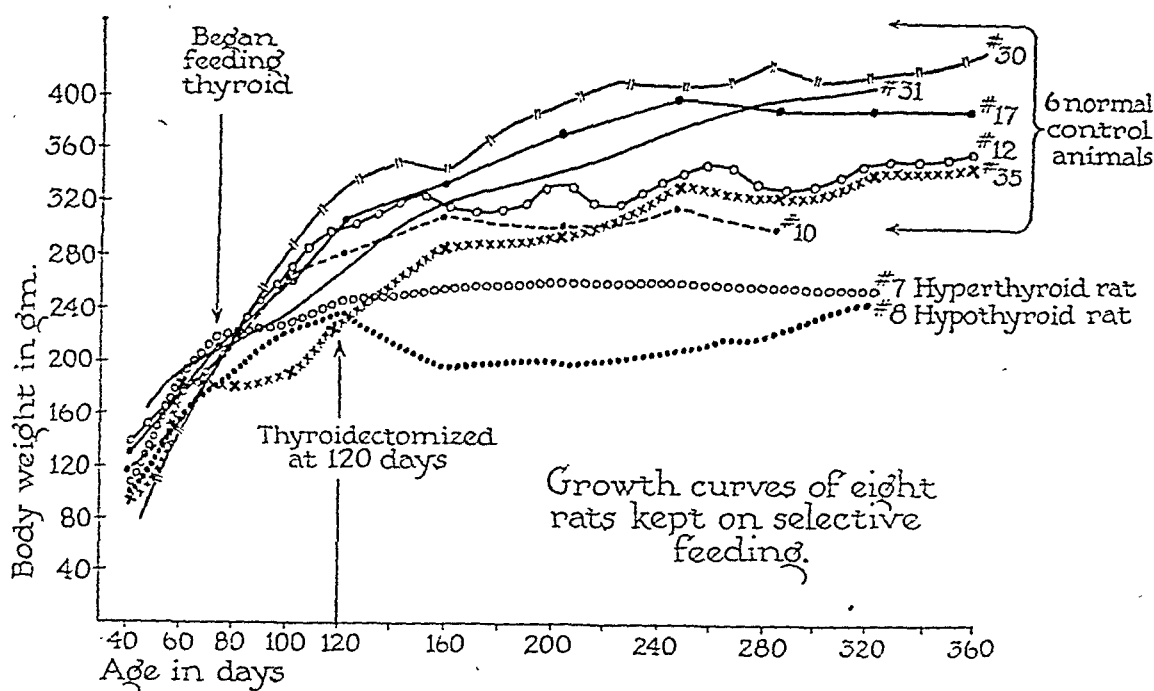
Fig. 3

TABLE 2

Average BMR's in cal./sq. m. body surface/24 hours

	BEFORE 120 DAYS (ALL ARE NORMALS HERE)	AFTER 120 DAYS
Straight normals..........	889	862
Hypothyroids..............	891 (normal period)	736 (hypo-period)
Hyperthyroids............	780 (normal period)	1060 (hyper-period)

we chose the age of 120 days as an arbitrary division between "young" and "old" rats, and made sure that all our comparisons took this factor into consideration.

4. *Basal metabolic rates.*   We used three groups of rats, with 6 rats in each group.   Up to the age of about 100 to 120 days, all 3 groups were normal animals.   After the age of about 120 days, we selected 6 healthy animals as normals (see growth curves in fig. 3), 6 others from our thyroidectomies as good hypothyroid rats, and the remaining 6 were our best hyperthyroid rats.   Table 2 presents average BMR's for these 3 groups; in any one group the *same animals*

studied before the age of 120 days are also used in data for the period after the age of 120 days.

It will be noted that the strictly normal group experienced a drop in BMR of 3 per cent after the age of 120 days. The hypothyroids were 17 per cent lower after thyroidectomy than before. Finally, the hyperthyroid group had a 36 per cent higher BMR after the feeding of thyroid tissue than before. The clinical symptoms of hypothyroidism and hyperthyroidism correspond quite closely to the BMR. While the hypothyroids seemed sluggish, the hyperthyroids often made themselves conspicuous by violently jumping around in

TABLE 3

Average grams of food eaten per rat per 100 grams body weight per 4-day period

FOOD ARTICLE	BEFORE 120 DAYS (ALL NORMAL HERE)			AFTER 120 DAYS (POST-OP., ETC.)		
	Straight normals	To be hypo-	To be hyper-	Straight normals	Hypo- thyroids	Hyper-thyroids
	grams	*grams*	*grams*	*grams*	*grams*	*grams*
Casein......	5	5	4	6	6	6.5
Dextrin.....	7	8	12.5	5	5	7
Lard........	3	2.5	1	1	1	2
Yeast.......	3	5	5.5	2	1.5	3
Salt.........	0.3	0.6	0.2	0.2	0.3	0.2
Total.....	18.3	21.1	23.2	14.2	13.8	18.7

TABLE 4

Average food intake of 3 groups of rats, in per cent, calculated on basis of weight of food in grams

FOOD ARTICLE	BEFORE 120 DAYS (ALL NORMAL HERE)			AFTER 120 DAYS (POST-OP., ETC.)		
	Straight normals	To be hypo-	To be hyper-	Straight normals	Hypo-thyroids	Hyper-thyroids
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Casein......	27	24	17	42.5	44	35
Dextrin.....	38	38	54	35	36	37
Lard........	17	12	4	7	7	11
.Yeast.......	16	24	24	14	11	16
Salt.........	2	2	1	1.5	2	1
Total.....	100	100	100	100	100	100

their cages. Several such hyperthyroid rats learned to pull our non-spill feeders apart.

5. *Composition of diets selected.* In reporting our data, we have converted all figures to apply per 100 grams of the animals' body weight, so that we can compare the feeding of young and of older animals. Table 3 shows the intake, in grams, averaged for the three groups of 6 rats each, and calculated for a 4-day period. For ease in further interpretation, the data in table 3 have been converted into percentages, as seen in table 4.

Finally, table 5 presents the percentages of *caloric* intake as protein, carbohydrate, and fat. Tables 3, 4 and 5 all contain data based on the same 18 rats.

DISCUSSION. An examination of tables 3, 4 and 5 yields several observations. (Note that data in table 3 are per 100 grams body weight.) It is seen from table 3 that there is a relative decrease in food intake with increasing age. This decrease amounted to 22 per cent in our normal rats, to 35 per cent in thyroidectomized rats, and to only 19 per cent in the hyperthyroid rats. Actually, these hyperthyroid rats ate a lot more food per 100 grams body weight than did either the normals or hypothyroids (table 3).

A decrease in appetite for lard with increasing age is seen in these tables, at least in the normal and hypothyroid rats. The increase in lard consumption by hyperthyroid rats is largely due to one rat, which ate much lard after being fed thyroid tissue.

These tables show an unexpectedly high protein intake. As seen in table 5, young rats selected 28 per cent to 32 per cent protein (this includes the protein fraction of yeast). Older rats even showed a marked increase in this protein

TABLE 5

The percentages of caloric intake as protein, carbohydrate and fat

DIETARY CONSTITUENT	BEFORE 120 DAYS (ALL NORMAL HERE)			AFTER 120 DAYS (POST-OP., ETC.)		
	Straight normals	To be hypo-	To be hyper-	Straight normals	Hypo-thyroids	Hyper-thyroids
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Protein....................	30	32	28	46	46	38
Carbohydrate...............	39	44	63	39	39	41
Fat........................	31	24	9	15	15	21

feeding, to the point where almost half their diet consisted of protein. This was not due to illness, because all animals reported here were consistently healthy, and the normals had a good coat of hair at all times. This experimental result raises a question as to why over 22 per cent protein should impair the appetite of white rats, as has been reported (9). The high intake of protein is the more remarkable in view of the apparent dislike rats have for casein. However, this is explained in part by the fact that some of the protein intake was in the form of yeast, which rats like.

The relatively high intake of yeast by these rats is interesting and contrary to earlier reports (10). For some 4-day periods certain rats averaged over 5 grams of yeast intake per day, and they not uncommonly ate 3 or 4 grams of yeast per day. Table 3 shows that the thyroidectomized animals demonstrated the greatest drop in yeast appetite.

The appetite of rats for salt mixture has varied somewhat in our experience with selective feeding, and has been greater in some groups of young rats than is shown here. However, older rats uniformly prefer a low salt intake, and they usually eat slightly less than 2 per cent salt in their selected diet (table 4).

The appetite of rats for dextrin resembles their appetite for lard in that these constituents are selected with peculiar variations. A rat may suddenly reverse

its selection from a low carbohydrate and high fat diet, to a low fat and a high carbohydrate diet; some weeks or months later another reversal of selection may occur (fig. 1). Such variable feeding on dextrin and lard is reflected in the variations among the three groups before the age of 120 days, when the rats are still all normal (tables 3 and 4). These observations resulted from our study of individual animals over a period of several months. Other studies in self-selection with rats have not been continued for such long periods of time (1, 4, 6).

In closing this discussion some comparison should be made between the diets selected by our rats and a well-known standard diet, such as that of Osborne and Mendel. We have found that the latter diet does not support good growth in rats after they have been raised on it to the age of 4 or 5 months. Possible deficiencies may be suggested by the comparison in table 6.

It is readily seen that the young rats select a diet more nearly like that of Osborne and Mendel than do the older rats. The low protein, high fat and salt content of the standard diet seem to make it unsatisfactory for older rats.

TABLE 6

Constituents of the standard Osborne and Mendel diet, of the self-selected diet of our 18 young rats, and of our 6 older rats, respectively

FOOD ARTICLE	OSBORNE AND MENDEL DIET	DIET SELECTED BY YOUNG RATS	DIET SELECTED BY OLD RATS
	per cent	*per cent*	*per cent*
Casein........................	18	22	42.5
Dextrin.......................	52	44	35
Lard..........................	24	10	7
Yeast.........................	3	22	14
Osborne & Mendel salt mixture..	3	2	1.5

CONCLUSIONS

1. Selective feeding, using relatively pure constituents, will support good health and growth in most rats for a period of over one year.

2. All food constituents should be kept in separate feeding cups, rather than having some mixed together (such as yeast and salt).

3. The diet selected by rats varies according to age; and the critical age dividing "young" and "old" rats is about 4 months.

4. Older rats eat less food per 100 grams body weight than do young rats.

5. Young rats select more fat and salt than do older rats, while the latter eat more protein. However, all our groups have eaten much more protein than is allowed in the Osborne and Mendel diet.

6. The alternate intake of fat and carbohydrate is so definite in some rats as to suggest that they "eat for calories."

7. Six rats were made hypothyroid, 6 others were made hyperthyroid, and 6 were kept as normal controls. The thyroidectomized rats showed a markedly greater decrease in food intake than did the normals, while the hyperthyroid rats ate much more food than either of the other two groups. No characteristic qualitative differences in food selection were noted due to thyroid abnormalities.

REFERENCES

(1) BARELARE, B. AND C. P. RICHTER. This Journal **121**: 185, 1938.

(2) DAVIS, C. M. Am. J. Dis. Child. **36**: 651, 1928.

(3) DAVIS, C. M. Canad. Med. A. J. **41**: 257, 1939.

(4) MITCHELL, H. S. AND L. B. MENDEL. This Journal **58**: 211, 1921.

(5) OSBORNE, T. B. AND L. B. MENDEL. J. Biol. Chem. **35**: 19, 1918.

(6) RICHTER, C. P. *et al*. See references in J. Pediat. **20**: 230, 1942, and this Journal **135**: 781, 1942.

(7) YOUNG, P. T. Psychol. Bull. **38**: 129, 1941 (a review).

(8) JONES, H. M. J. A. M. A. **115**: 274, 1940.

(9) HAMILTON, T. S. J. Nutrition **17**: 565, 1939.

(10) JAKWAY, I. J. Comp. Psychol. **26**: 157, 1938.

DEPRESSIVE EFFECTS PRODUCED ON APPETITE AND ACTIVITY OF RATS BY AN EXCLUSIVE DIET OF YELLOW OR WHITE CORN AND THEIR CORRECTION BY COD LIVER OIL[1]

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In terms of the actual work output of individual organisms very little is known regarding the energy value of various foodstuffs. In the series of which the present experiment forms a part an effort is being made to throw more light on this phase of the nutritive value of foods.

In previous experiments measurements were made of the spontaneous work output (revolutions of a drum) of rats kept on a diet in which dextrose constituted the sole source of nourishment (1). On this diet the rats lived an average time of 36 days and did not show any signs of specific nutritional deficiencies, though they were markedly emaciated. Of special interest in the present connection is the fact that the rats remained very active, averaging 10 to 12 miles per day in the revolving drum for the first 20 days. When given access to a 0.02 per cent solution of thiamine hydrochloride the rats on dextrose lived slightly more than twice as long, averaging 74 days. The only definite deficiency symptom shown during this time was a constant cornification of the cells of the vaginal smears, after an average time of 51 days, presumably indicating a lack of vitamin A. Here again the rats remained very active, averaging 6 to 8 miles per day up to 60 days on the diet. Clearly, dextrose, either alone or supplemented with thiamine hydrochloride, serves as an excellent source of energy. Similar studies have been made using maltose, sucrose, levulose, galactose and lactose (2, 3).

In the present experiments this same technique has been applied to the study of the energy value of white and yellow corn. Measurements of activity have been made, as well as observations on the effects produced on appetite and water intake, body weight, vaginal smear cycles, and general health.

METHODS. · Eight female rats were placed in separate activity cages at an average age of 45 days; for the first 15 days they were given free access to our stock diet and tap water. At 60 days of age and an average body weight of 135 grams, these rats were placed on an exclusive diet of either freshly ground yellow or white corn[2] and tap water. Eighty-five days later they were given access also to cod liver oil. Another 40 days later, that is at an average age of 185 days, they were killed and autopsied.

[2] The yellow hybrid corn g-94 and the white hybrid corn g-527W were kindly furnished by Funk Brothers Seed Company.

The cages were composed of two parts: a living compartment 11 x 3½ x 5 inches and a revolving drum 12½ inches in diameter and 6 inches wide, separated by a metal partition with an opening 3 inches in diameter. The living compartment, which contained a food cup with a shield built to eliminate all spillage, and a 100 cc. graduated inverted water bottle, was made as small as possible in order to force the rat to expend most of its energy in the drum, where it could be measured by means of a cyclometer. The cod liver oil was later offered in an added 30 cc. graduated bottle.

For the reason that coprophagy may play a particularly important part in single food choice experiments, special precautions were taken to prevent easy access to the feces. In the living compartment, a ⅓ inch wire mesh bottom permitted the feces to drop through freely to a sawdust pan several inches below and beyond the reach of the rat. In the revolving drum, which was made of ¼ inch wire mesh, a space of ¼ inch between the drum and the central partition permitted the feces to drop through freely to the pan, well beyond the rat's reach. The results of previous experiments have shown that this method limits coprophagy sufficiently to give quantitatively reproducible results (1).

Records were made daily of running activity, food and water intake, and vaginal smears, and weekly of body weight. The rats were carefully examined at weekly intervals for specific signs of nutritional deficiency.

RESULTS. *Yellow corn.* Figure 1A gives the record of one of the 4 rats on yellow corn. The abscissae show age in days, the ordinates running activity in revolutions of the drum, food intake and body weight in grams. This rat was placed in the activity cage on our stock diet at an age of 44 days, changed to the yellow corn diet at 62 days, and given access to cod liver oil at 146 days of age. During the last 5 days on the stock diet the daily running activity reached an average level near 24,000 revolutions and food intake a level near 16 grams. On the yellow corn diet activity remained on essentially the same level for 20 days, then decreased, first to a level near 8,000 revolutions per day, and finally, between the 136–146th day period, to a level around 1,000 revolutions per day. For the first 10 days the intake of corn approximately equalled the previous intake of stock diet. Thereafter, up to the 146th day, the intake decreased at a slow but steady rate, finally reaching a level near 7 grams per day. Body weight increased slightly during the 80 days on the corn diet. Vaginal smears (cornified cells shown at the top of the chart) showed regular 4 to 5 day cycles for the 15 days on the stock diet and for the first 20 days on the corn diet. Thereafter the cycles became irregular for 20 days and then disappeared altogether, leaving only a diestrous picture of leucocytes and nucleated epithelial cells. Otherwise the rat did not show signs of any specific nutritional deficiency at this time. Cod liver oil offered on the 146th day was taken in moderate amounts for the first day, then only in minimal amounts. The cod liver oil ingestion had immediate and profound effects on activity, corn intake, body weight and vaginal smears. Within 18 days the average daily activity had increased from a level around 1,000 revolutions to 22,000 revolutions (13.5 miles), approximately the same as that on the stock diet; the average daily corn intake increased from

7 to 14 grams, and the body weight from 140 to 174 grams; within 4 days the vaginal smears again showed regular 4 day cycles of cornification, coinciding with the peaks of running activity.

Table 1A summarizes the results of the observations made on the 4 rats on yellow corn. The average daily activity decreased from 19,969 revolutions for the first 10 days on the corn diet to 2,139 revolutions for the 70–80th day period, and increased again to 15,803 for the 30–40th day period on cod liver oil. All 4 rats became more active within 3 or 4 days after the addition of the cod liver oil. The average daily corn intake decreased from 15.4 grams for the first 10 days to 7.5 grams for the 70–80th day period, and increased again to 11.3
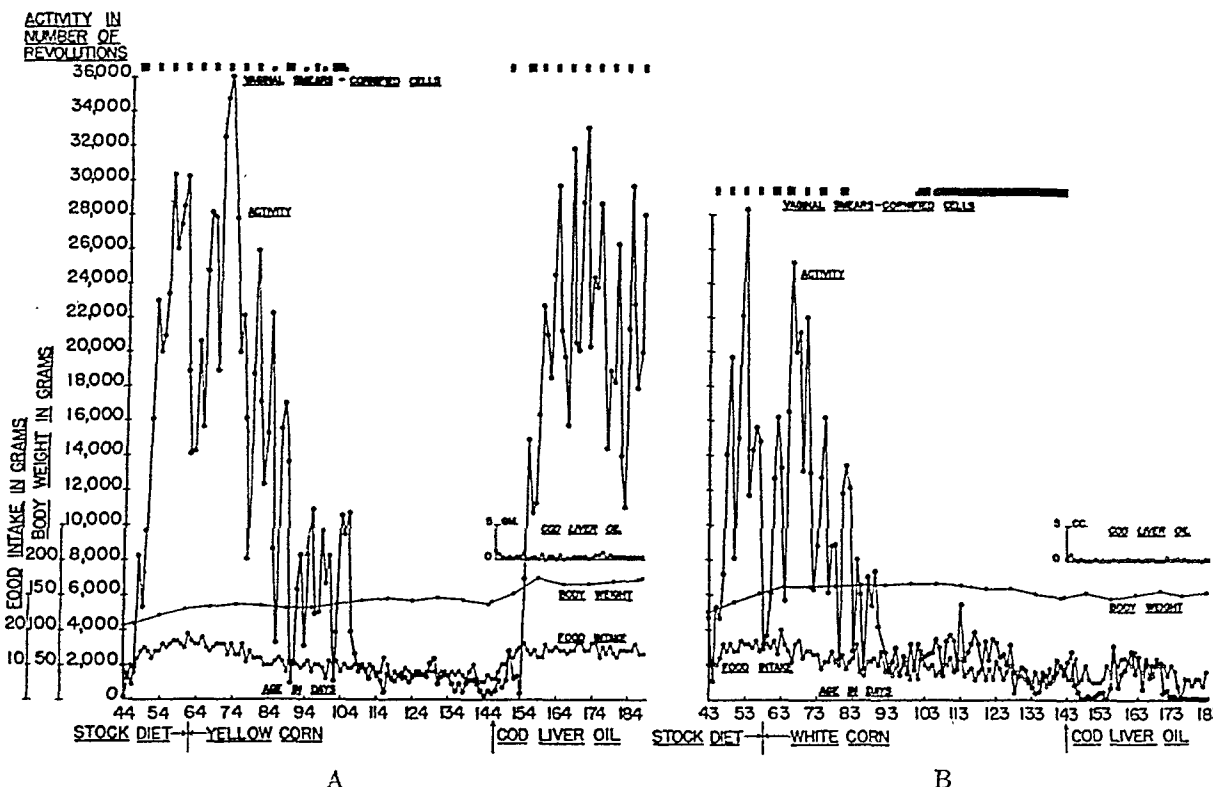
Fig. 1

grams for the 30–40th day period on cod liver oil. All four rats showed a detectable increase in food intake after the second day on cod liver oil. Body weight increased from an average of 132 grams at the start of the corn diet to 143 grams on the day cod liver oil was started. (During the same interval control rats on the stock diet increased to 189 grams.) At the end of the 40 day period on cod liver oil the average body weight had increased to 168 grams.

Figure 2 summarizes the effects produced on vaginal smears, showing only the cornified cells. All 4 rats on yellow corn showed quite regular 4 to 5 day cycles for the 15 days on the stock diet and for the first 20 days on corn. From then on, until the cod liver oil was offered, the cornified cells gradually disappeared from the smears, leaving only leucocytes and epithelial cells. Within 4 days of

the addition of cod liver oil, 3 rats showed their first day of cornified smears, the fourth showed it 4 days later. Thereafter during the 40 day period all 4 rats, with the exception of one rat which missed 2 cycles, showed 9 regular 4 day cycles. It is also noteworthy that the peaks of running activity coincided as they do in normal animals with the days of cornification.

Just prior to the addition of the cod liver oil 3 of these animals were reported in excellent condition and the fourth was in good shape except for slight rusting of the back of the head and neck. When they were killed, 40 days later, their appearance differed little from that of normal controls. Body weight averaged only 21 grams less, eyes were large and clear, teeth were normal, paws and tails were clean; however, the hair was yellow and matted over the hind quarters of all 4 animals. At autopsy there were no gross deviations from the normal, except a complete absence of thymus tissue, whereas this gland weighed an average of 176 mgm. in normal controls. As seen in table 2A, the weights of the other endocrine glands all fell within normal limits, with the exception of an unexplained increase in size of the ovaries, which averaged 42 and 40 mgm. for
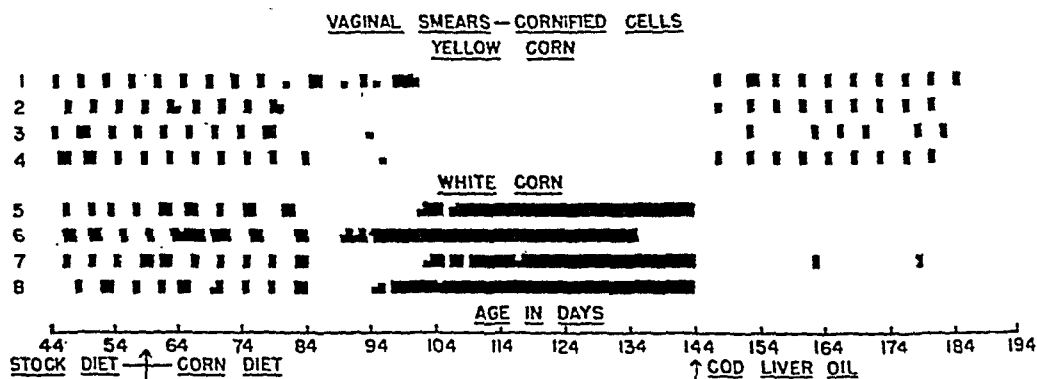
Fig. 2

the left and right glands respectively, as compared with 36 and 32 mgm. in the controls.

White corn. Figure 1B gives the record of one of the 4 rats on the single food choice of white corn. For the first 100 days of the experiment, that is, until the cod liver oil was added, the activity, food intake, and body weight records are essentially the same as those of the rat on yellow corn shown in figure 1A. The vaginal smears, however, differed markedly. In contrast to the constant diestrous smears of the rat on yellow corn, the smears of this rat showed cornified cells and no leucocytes or epithelial cells for the last 35 days before cod liver oil was added. Also in contrast with the yellow corn-fed rats, the cod liver oil, which was taken in minimal amounts, had no effect on activity, only a temporary if any effect on food intake, and body weight showed only a very small increase. A most striking effect was produced, however, on the vaginal smears. Within 24 hours all cornified cells disappeared, leaving only epithelial cells and leuco-cytes, but still giving no evidence of cyclic changes.

Table 1B summarizes the results of the observations made on the 4 rats on

white corn.[3]   On the corn diet the average daily activity of the 4 rats decreased from 16,203 revolutions for the first 10 days to 2,116 for the 70–80th day period. On the cod liver oil one of the surviving 3 rats became slightly more active again, while the other two became progressively more inactive.   The average daily intake of white corn decreased from 14.8 to 5.5 grams.   On the cod liver oil 2 rats showed an increased, one a decreased food intake.   The average body weight decreased from 138 grams on the first day of the corn diet to 126 grams on the 83rd day.   On the cod liver oil one rat showed a marked increase in weight and one a moderate increase, while one maintained its weight.   Figure 2 shows that the cornified cells disappeared from the vaginal smears on the day after cod liver oil was made available.   Thereafter only one animal showed any cornified cells, then only on 2 days.   Thus, the records of the yellow corn-fed rats before the addition of cod liver oil were very similar to those of the white corn-fed group after the oil was added.

Before the cod liver oil was added, 3 of the 4 animals showed deficiency symptoms of eyes, teeth or hair, and when they were killed 40 days later they were very clearly distinguishable from the normal controls and from the yellow corn-fed rats, mainly by their smaller size, but also by a marked yellowing and roughening of the coat, by their spectacle eyes, and by the fact that all except one had poor teeth: in 2 cases both upper incisors were broken off and the lowers were abnormally long.   Autopsy revealed no gross abnormalities except the complete absence of thymus tissue noted also in the yellow corn-fed group, but all endocrine glands were found to be markedly smaller than those of normal controls.   In particular, whereas the thyroids, adrenals, and ovaries were decreased approximately in proportion to the lower body weight, the uteri were found to be relatively far smaller, weighing approximately 20 per cent as much as those of the controls.

DISCUSSION.   Corn is a cereal grain of such great agricultural importance that it is only natural that it should have been thoroughly studied from a nutritional standpoint.   It has long been known that zein, the principal corn protein, is deficient in the amino acids, tryptophane, tyrosine and lysine (4, 5).   In addition, corn is known to be a poor source of calcium (6).   In 1919 Steenbock compared the nutritive value of various species of corn, and pointed out the higher vitamin A content of the yellow varieties (7).   The vitamin B complex, on the other hand, is apparently present in adequate amounts for normal growth in both yellow and white varieties (8).   Since pellagra is so very common in regions where corn constitutes a large proportion of the diet, and since a deficiency of nicotinic acid is known to play a major rôle in the production of this disease syndrome, it is not astonishing to find that corn is a poor source of this vitamin (9).   Steenbock and Coward also pointed out that the vitamin D content of corn is low (10).   Apparently, like other cereal grains, it is relatively rich in vitamin E (11).

However, when rats were kept on an exclusive diet of either white or yellow corn from 60 to 145 days of age, none of these deficiencies became sufficiently

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[3] One of the rats died after 76 days on the diet.

TABLE 1

RAT NO.	AVERAGE DAILY RUNNING ACTIVITY IN REVOLUTIONS OF DRUM			AVERAGE DAILY CORN INTAKE IN GRAMS			BODY-WEIGHT IN GRAMS		
	First 10 days on corn diet	70–80th days on corn diet	30–40th days on cod liver oil	First 10 days on corn diet	70–80th days on corn diet	30–40th days on cod liver oil	At start of corn diet	At end of 80 days on corn diet	At end of 40 days on cod liver oil
A. Yellow corn									
1	23,162	1,024	22,201	16.2	7.7	14.0	134	140	174
2	14,255	1,241	17,872	13.7	7.3	10.0	124	146	157
3	22,560	4,401	14,816	14.9	8.2	9.5	122	142	162
4	19,898	1,889	8,324	16.6	6.9	11.7	147	145	178
Average...	19,969	2,139	15,803	15.4	7.5	11.3	132	143	168
B. White corn									
5	12,369	1,287	380	15.4	5.9	7.3	152	150	151
6*	23,734	2,371		15.7	4.7		135	114	
7	16,069	1,969	5,964	13.5	5.8	10.1	132	116	174
8	12,640	2,837	1,117	14.4	5.5	5.0	133	124	144
Average ..	16,203	2,116	2,487	14.8	5.5	7.5	138	126	156

* Animal died after 76 days on white corn diet.

TABLE 2

Autopsy findings

Endocrine weights in milligrams

RAT NO.	BODY WEIGHT	ADRENAL LEFT-RIGHT	UTERUS	OVARIES LEFT-RIGHT	THYROID	THYMUS	TEETH
A. Yellow corn							
	grams						
1	174	35–30	411	48–49	11	Absent	Good condition
2	157	34–28	348	35–32	16	Absent	Good condition
3	162	24–26	231	38–37	14	Absent	Good condition
4	178	25–15	421	45–42	14	Absent	Good condition
Average............	168	29–25	353	42–40	14		
B. White corn							
5	151	25–23	131	28–38	16	Absent	Upper teeth broken off
7*	174	20–24	182	30–34	16	Absent	Poor condition
8	144	13–14	92	15–11	11	Absent	Poor condition
Average............	156	19–20	102	24–28	14		
C. Regular food							
Controls 150 days .	189	26–26	498	36–32	17.4	176	Good condition

* Animal 6 died after 76 days on the white corn diet and no autopsy was obtained.

marked to be obvious unless records were kept of the activity and vaginal smears. Thus, in both groups, the weight was essentially maintained though there was little or no growth; there were no evidences of dermatitis and no gross skeletal changes. Only during the last quarter of the period did the white corn-fed rats begin to show the spectacle eyes and roughening of the fur characteristic of an early vitamin A deficiency, as well as poor occlusion of the incisors. On the other hand, during the first 25 days of the diet both groups of animals showed a loss of spontaneous activity of nearly 70 per cent, and the vaginal smear cycles were lost: the white corn-fed rats showed persistent cornification, while the yellow corn-fed animals went into a state of constant diestrous.

But the striking difference between the two groups was only brought out when cod liver oil was made available. Whereas this addition had little or no obvious effect on the food intake, body weight, and activity of the white corn-fed rats, it produced in them an almost immediate change in the vaginal mucosa from a state of constant cornification to one of constant diestrous. In contrast, the yellow corn-fed rats showed a marked general improvement almost immediately following the ingestion of the cod liver oil: first the food intake and body weight began to increase, then normal 4 day estrous cycles reappeared, and later (approximately 7 days after the oil was added) the activity increased abruptly.

It is not clear at this time what factors in the cod liver oil are responsible for the various changes: both vitamins A and D must be taken into consideration, as well as the various fatty acids, sterols, and other, perhaps unknown, constituents. The fact that the yellow corn-fed rats returned to normal cycles of vaginal cornification, and gained weight, and became more active when cod liver oil was added to their diet, raises the question whether all three changes were brought about by the same factor or whether there were a number of substances involved. The data so far available are not sufficient to answer this question.

On the assumption that for the purposes of relatively short term experiments, yellow corn is lacking primarily in one substance, one can postulate further concerning the deficiencies of white corn. In the first place, there is almost certainly a deficiency of vitamin A, which is corrected by the fish oil supplement. In the second place, it seems likely that the white corn is deficient also in the same substance lacking in yellow corn and supplied by cod liver oil, whether this is vitamin D or some other factor. Further, since the addition of cod liver oil to the diet of the white corn-fed rats still leaves them in a state of obvious nutritional deficiency, as indicated by the limited appetite and growth, inactivity, diestrous smears and general appearance, there would seem to be lacking in the white corn some factor present in yellow corn and not supplied by cod liver oil.

SUMMARY

1. Female rats on an exclusive yellow corn diet for a period of 85 days showed a gradual loss of appetite, a scarce maintenance of starting body weights, a great loss of activity, and development of a diestrous condition of the vaginal mucosa. They did not, however, show any other signs of specific nutritional deficiency in this time.

2. Female rats on an exclusive white corn diet for a period of 85 days showed a similar picture, with the exception that after 45 days the cells of the vaginal smears became constantly cornified, and after about 76 days the upper teeth became worn and the lower teeth overgrown. They also showed deficiency symptoms of the eyes and hair.

3. Cod liver oil offered the rats on the yellow corn diet was taken in moderate amounts for the first day and then in minimal amounts, resulting in an almost immediate increase in appetite, body weight and activity. Regular 4 day cycles also reappeared in the vaginal smears within 4 days. Within 20 days the rats had practically reached their original high running levels.

4. Cod liver oil offered the rats on the white corn diet was taken in minimal amounts, producing almost no effect on activity, a small effect on appetite, and only a slight increase in body weight. Within a day, however, the vaginal smears were changed from a condition of constant cornification to one of constant diestrous.

5. In white corn there is almost certainly a deficiency of vitamin A, as well as of the factor lacking in yellow corn, both of which are corrected by the cod liver oil supplement. Moreover, there is evidence to indicate that yellow corn contains some factor lacking in white corn and not present in cod liver oil.

REFERENCES

(1) RICHTER, C. P. AND K. K. RICE. This Journal 137: 573, 1942.
(2) RICHTER, C. P., L. E. HOLT, JR. AND B. BARELARE, JR. This Journal 122: 734, 1938.
(3) RICHTER, C. P. AND K. K. RICE. Unpublished data.
(4) WILLCOCK, E. G. AND F. G. HOPKINS. British J. Physiol. 35: 88, 1906–07.
(5) OSBORNE, T. B. AND L. B. MENDEL. Jr. Biol. Chem. 17: 325, 1914.
(6) HOGAN, A. G. J. Biol. Chem. 29: 485, 1917.
(7) STEENBOCK, H. Science 50: 352, 1919.
(8) CROLL, H. M. AND L. B. MENDEL. This Journal 74: 674, 1925.
(9) GOLDBERGER, J., G. A. WHEELER, R. D. LILLIE, AND L. M. ROGERS. U. S. Pub. Health Reports 43: 1385, 1928.
(10) STEENBOCK, H. AND K. H. COWARD. J. Biol. Chem. 72: 765, 1927.
(11) SURE, B. J. Biol. Chem. 62: 371, 1924.

THE EFFECT OF URANIUM POISONING ON PLASMA DIODRAST CLEARANCE AND RENAL PLASMA FLOW IN THE DOG[1]

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The value of the clearance method in the study of renal physiology is now clearly recognized. The validity of the inulin clearance (C_I) as a measure of glomerular filtration and of the diodrast clearance (C_D) as a close approximation to the plasma flow to the active secretory and excretory portions of the normal kidney (effective plasma flow) has been established for several species, chiefly the dog and man. The uninephrectomized dog with the remaining kidney explanted is a particularly satisfactory subject for studies of clearance and renal blood flow (RBF) or plasma flow (RPF) since the latter may be calculated directly from the clearance and arterio-venous difference of any test substance. This procedure has been used in the study of the relation between C_D and RBF in the normal dog kidney (White, 1940a; Corcoran et al., 1941) and in the damaged kidney (White, 1940b; White et al., 1941).

In a previous paper (Hayman et al., 1939), the effect of uranium on the dog kidney was discussed and data were presented to show that the decrease in C_I and creatinine clearance (C_C) was not due to a decrease in RBF. The low clearances and extractions seemed most satisfactorily explained, considering the histological as well as the analytical data, on the basis of back diffusion of inulin (I) and creatinine (C) in the damaged tubules. The greater decrease in C_C than in C_I was presumed to be the result of the greater back diffusion of the smaller C molecule. During the course of a study of the relation between C_D and RBF in the dog kidney damaged by a variety of means, uranium poisoning has been reinvestigated and the method has been extended to include the use of diodrast (D) as a test substance.

METHODS. Trained dogs were used, maintained on the usual kennel rations including meat. One kidney was explanted to make the renal vein accessible for venipuncture. Following recovery from this operation, the other kidney was removed. Experimental studies were begun no sooner than three months following the second operation.

Two control experiments were performed on each dog before it was given a subcutaneous injection of 2 mgm. of uranium acetate per kilo of body weight. Following the administration of uranium, the experiments were repeated at the height of the effect (about five days) and at varying intervals thereafter until

substantial recovery had occurred. One dog, no. 4, was sacrificed after the five-day period for histological examination of the kidney.

Clearance and extraction data were obtained as follows. An intravenous infusion of normal saline solution was started and maintained throughout the experiment at a rate of about 2 cc. per minute to insure adequate urine flow. C, I and D in saline solution were injected subcutaneously in two sites in a total volume of 30–50 cc. For control experiments, 0.1 gram of C, 0.2 gram of I, and 0.175 gram of D (0.5 cc. of 35 per cent solution) per kilo of body weight were used. Following kidney damage, these quantities were reduced one-half.

Fifteen minutes following the injection, the bladder was emptied and washed with normal saline solution through an in-dwelling catheter. Three 20-minute urine collections were made, the bladder being washed with two 10 cc. portions of saline solution at the end of each period. Blood samples were taken from the jugular vein ("arterial") and renal vein ("venous") as near to the mid-points of the urine collection periods as possible. These periods were used for D clearances only. The plasma D level during this 60-minute interval remained very nearly constant at a level of about 5 mgm. per 100 cc. or less. D was determined in arterial and venous whole blood, arterial plasma and urine, giving data for the calculation of both whole blood and plasma C_D, whole blood extraction and RBF.

Following these three periods, 1.5–2.0 cc. of 35 per cent D solution per kilo of body weight were injected intravenously. Beginning 15 minutes later, four additional clearance periods were obtained as above. Arterial plasma was analyzed for D, I and C, venous plasma for I and C, urine for D, I, C and urea (U). The pooled remainders of arterial plasma were utilized for albumin and U determinations. The D, I, C and U plasma clearances, RPF (using both I and C extractions) and Tm were then calculated from the data. Other calculations are explained in the discussion. Hematocrit readings were obtained on a sufficient number of blood samples to make possible the interconversion of RPF and RBF.

During this 80-minute interval, the plasma I level remained very nearly constant at 5–10 mgm. per 100 cc. The plasma C level fell slowly, the D level more rapidly but the rate of fall in both cases was so nearly linear that no significant error was introduced by assuming the interpolated plasma concentration at the mid-point of the urine collection period to be equal to the mean concentration throughout the period.

Diodrast was determined in both plasma and whole blood by the method of Alpert (1941) with the application to the whole blood filtrate analyses of a correction factor based on the hematocrit reading (Bobey and Price, 1942). Inulin was determined by the method of Alving, Rubin and Miller (1939), using the Cenco-Sheard spectrophotelometer for the color readings. Creatinine was determined in Folin-Wu filtrates as the alkaline picrate, by a standard method slightly modified for use with the Spectrophotelometer. Plasma urea was determined by the method of Van Slyke and Kugel (1933), urine urea by Van Slyke's urease method (1937). Albumin was calculated as N × 6.25 after Kjeldahl analysis of the globulin-free filtrate obtained with 22 per cent sodium sul-

TABLE 1

(1) DOG	(2) TIME AFTER URANIUM	CLEARANCE (3) Inulin plasma	(4) Creatinine plasma	(5) Urea plasma	(6) Diodrast plasma	(7) Diodrast blood	EXTRACTION RATIO (8) Inulin plasma	(9) Creatinine plasma	(10) Diodrast blood	(11) AVERAGE RPF	(12) AVERAGE RBF	(13) C_D/RPF $\frac{(7)}{(11)}$	(14) T_m	(15) TUBULAR C_D	(16) TUBULAR EXTRACTION $\frac{(15)}{(11)}$
		cc./min.	cc./min.	cc./min.	cc./min.	cc./min.				cc./min.	cc./min.		mg.I/min.	cc./min.	
1	Control	33.5	31.9				0.322	0.254		115	191				
	5 days	7.7	5.7				0.077	0.053		105	173				
	7 weeks	19.4	15.8				0.221	0.195		85	151		2.9		
	14 months	32.4	32.0	22.1	78	96	0.275	0.325	0.607	105	162	0.74	5.1	51.0	0.49
2	Control	47.8	47.8	27.2	128	148	0.300	0.293	0.611	162	254	0.79	7.2	86.0	0.53
	5 days	18.1	15.2	11.9	13.8	15.5	0.077		0.055	214	310	0.06	-1.0	-2.4	-0.01
	12 days	24.1	22.0	16.1	44.4	52.4	0.190	0.167		130	186	0.34	1.7	23.0	0.18
	6 weeks	36.9	36.9	23.8	81.0	93.0	0.263	0.276	0.523	134	193	0.60	4.8	51.7	0.39
	3 months	40.5	39.7		109.0		0.321	0.264		140	226	0.78		73.5	0.53
3	Control	45.7	40.1	23.8	169.0		0.227	0.204		199	325	0.85	15.8	129.0	0.65
	5 days	14.6	8.1	6.4	14.4		0.057	0.078		180	300	0.08	-2.5	1.6	0.01
	10 days	15.3	10.6	7.6	66.0		0.122	0.070		140	228	0.47	2.7	52.6	0.38
	1 month	29.0	27.3	18.6	130.0		0.224			132	200	0.98	13.9	104.0	0.79
4	Control	38.8	40.4	26.6	105.0	124.0	0.263		0.681	135	213	0.78	7.7	74.0	0.55
	5 days	13.6	11.0	9.4	9.7	10.9	0.095	0.087	0.040	153	213	0.07	-0.4	-2.6	-0.02

Diodrast clearances were not done in the first two experiments or at low plasma levels in the third experiment with dog 1.

phate.   Free diodrast (not bound to albumin) was determined from a nomogram constructed from the data of Smith and Smith (1938).

RESULTS.   The determined clearances and blood flows and calculations based on these data are summarized in table 1.   Each control represents the average of 6–8 clearance periods in two experiments; the data following uranium poisoning are averages of 3–4 clearance periods in single experiments.

The plasma flow (column 11) is the average obtained from creatinine and inulin plasma clearances and extractions and from whole blood diodrast data and hematocrit values except in a few instances where one method gave a value inconsistent with the other two.

Column 13 shows the extent to which C_D approximates the independently measured plasma flow.   This value falls from an average of 81 per cent in the control periods to 7 per cent following uranium.

Despite the fact that C_I is probably no longer an accurate measure of glomerular filtration because of back diffusion of inulin in the damaged tubules, it may nevertheless be used as a minimum measure of this function.   On this basis the "tubular D plasma clearance" as defined by White (1940b) may be calculated and the ratio of this value to RPF taken as the "tubular extraction", a measure of the fraction of RPF cleared of D by tubular activity.   Tubular D clearance (column 15), following uranium administration, falls to practically zero in one case and to a negative value in the other two, evidence of complete suppression of tubular D secretion.   The tubular extraction (column 16) falls from an average of 0.58 to a negative value.   The effect of uranium on the maximum rate of tubular excretion of D (Tm) is shown in column 14.

In charts 1 and 2 the output of diodrast per minute and the plasma diodrast clearance respectively are plotted against the plasma diodrast level using the data obtained in the individual clearance periods both before and five days after uranium poisoning.

DISCUSSION.   The changes in C_I and C_D following uranium poisoning are consistent with those previously reported (Hayman et al., 1939).   Both are reduced, the latter to a greater extent.   The urea clearance is also reduced and approximately parallels C_I.   The diodrast clearance, however, is reduced to a much greater extent in all cases falling below C_I (columns 3–7).

Coincident with the reduction in clearances is a parallel reduction in extractions (columns 8–10) so that, in the acute stage of uranium poisoning, there is no significant change in RPF (column 11).   In two of the animals there was a subsequent slight, possibly permanent, lowering of RPF.

The principal effect of uranium poisoning observed histologically is tubular damage.[3]   In the absence of evidence of marked glomerular change, it seems reasonable to explain the reduction in C_I and C_C on the basis of extensive tubular back diffusion of these substances.   The behavior of C_D further strengthens this

[3] Dog 4 was sacrificed six days after poisoning and kidney sections were examined histologically by Dr. H. Goldblatt.   The findings were similar to those described by MacNider (1924) and summarized briefly by Hayman et al. (1939), namely, marked tubular necrosis with very slight glomerular involvement.

hypothesis. The tubular component of D extraction is eliminated in the acute state of poisoning when the most marked tubular damage is apparent. Diodrast clearance is reduced to the level of C_I and C_C and D is thus being removed from the blood by the same mechanism as are I and C. Actually C_D, like C_C, is lower than C_I presumably indicating that not only has D secretion been abolished but also that back diffusion of a portion of the glomerular output has occurred. Since both C and D (by comparison with I) apparently diffuse back into the circulation across the damaged tubular membrane, it is quite possible that the reduction of C_I as well as of C_C and C_D may be largely due to this process rather than to glomerular damage.

The reduction of tubular D plasma clearance and tubular D extraction to zero or negative values is a more striking presentation of the fact that tubular secretion of D is absent and may even be replaced to some extent by back diffusion of D. The fact that Tm, a measure of the rate of tubular excretory function independent of glomerular activity, falls to a negative value after poisoning can only be interpreted as additional evidence of complete replacement of tubular secretion by some tubular back diffusion.

The use of C_D as a measure of effective RPF is dependent on the ability of the tubule cells to remove essentially all of the D presented to them by the blood. The complete loss of this function resulting from uranium poisoning invalidates the use of C_D as an index of RPF and leads to impossibly high values for the filtration fraction (C_I/C_D). This suggests that any observed high value of the filtration fraction should be interpreted with the possibility in mind that it may be indicative of tubular dysfunction and may not necessarily be due to constriction of the efferent glomerular arterioles.
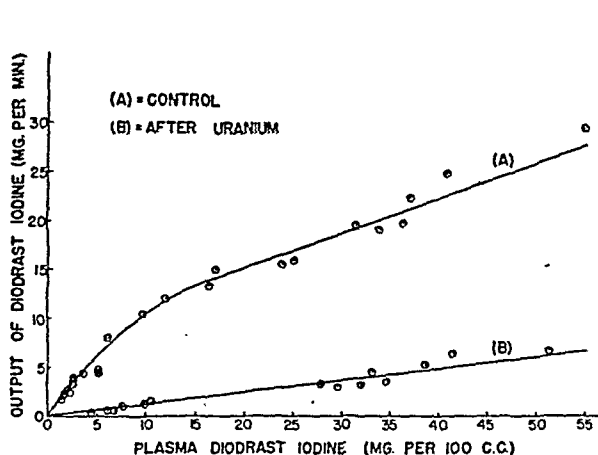
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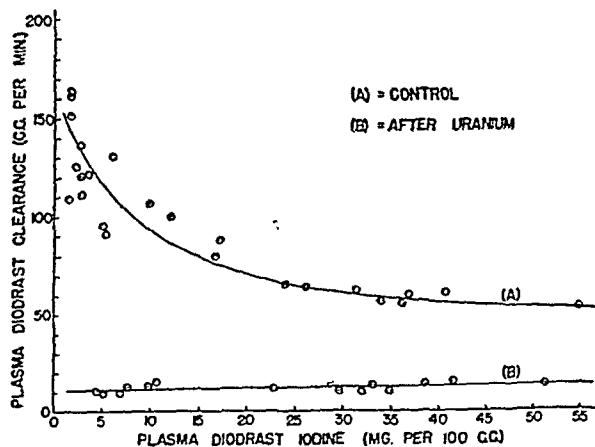
Chart 2

Chart 1. The relation of diodrast iodine output to plasma level before and after uranium poisoning.

Chart 2. The relation of plasma diodrast clearance to plasma diodrast iodine level before and after uranium poisoning.

In the control curve of chart 1 the output of D increases rapidly with increasing plasma level until at about 15 mgm./100 cc., the tubular capacity for D excretion

is saturated. Beyond this level the output increases linearly with plasma level and reflects only an increase in the amount filtered by the glomeruli. After uranium, the output is a linear function of plasma level with no initial rapid rise at low levels since only glomerular activity is involved. The slope of this line is less than the slope of the straight line portion of the control curve due to the apparent lowering of the level of glomerular filtration resulting from back diffusion in the damaged tubules.

As shown in chart 2, C_D in the normal kidney steadily decreases as the plasma D level is raised and the tubular mechanism for excretion becomes saturated, but following uranium poisoning tubular secretion of D is abolished and this relationship does not appear. Diodrast clearance following poisoning is low even at low plasma levels due to back diffusion of D from the glomerular filtrate but it is not further depressed as plasma levels are raised which is in sharp contrast to the control data.

As recovery from poisoning takes place, all clearances return toward normal values. The rate of improvement in the early stages is greatest in the case of C_D. Improvement in kidney function, as shown by the increase in C_I and C_C as well as by the return of concentrating ability and the disappearance of albuminuria and glycosuria which is observed in these dogs, coincides with recovery from tubular damage as indicated by the return of C_D toward normal levels.

CONCLUSIONS

1. The injury to the dog kidney produced by uranium causes a decrease in all clearances but negligible changes in the renal plasma flow. Diodrast plasma clearance is reduced to the level of inulin clearance or lower.

2. The reduction of diodrast plasma clearance to the level of apparent glomerular filtration is the result of the complete loss of the ability of the tubules to secrete diodrast.

3. The reduction in glomerular filtration is probably due in large part to back diffusion in the damaged tubules rather than to glomerular damage per se.

4. The use of diodrast plasma clearance as a measure of renal plasma flow and of inulin clearance as a measure of glomerular filtration is not justified in the uranium damaged kidney.

REFERENCES

ALPERT, L. K.   Bull. Johns Hopkins Hospital **68**: 522, 1941.
ALVING, A. S., J. RUBIN AND B. F. MILLER.   J. Biol. Chem. **127**: 609, 1939.
BOBEY, M. E. AND J. W. PRICE.   Proc. Soc. Exper. Biol. and Med. **51**: 217, 1942.
CORCORAN, A. C., H. W. SMITH AND I. H. PAGE.   This Journal. **134**: 333, 1941.
HAYMAN, J. M., JR., N. P. SHUMWAY, P. DUMKE AND M. MILLER.   J. Clin. Investigation
        18: 195, 1939.
SMITH, W. W. AND H. W. SMITH.   J. Biol. Chem. **121**: 107, 1938.
VAN SLYKE, D. D.   J. Biol. Chem. **73**: 695, 1927.
VAN SLYKE, D. D. AND V. H. KUGEL.   J. Biol. Chem. **102**: 489, 1933.
WHITE, H. L.   This Journal **130**: 454, 1940a.
        This Journal **130**: 582, 1940b.
WHITE, H. L., P. HEINBECKER AND D. ROLF.   Proc. Soc. Exper. Biol. and Med. **46**: 44, 1941.

INTRAMUSCULAR PRESSURE DURING LIFE AND AFTER DEATH[1]

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Henderson's concept of the existence of a venopressor mechanism postulates that venous pressure and venous flow are dependent on muscle tonus. When muscle tonus fails, venous pressure and venous flow must fail; thus, in shock, the tonus of the muscles lessens and collapse of the peripheral circulation ensues. Shock according to Henderson's concept is "hypotonia" (1).

In 1936 Henderson (2) devised an instument for the measurement of intramuscular pressure. A modification of it as proposed in 1936 by Kerr and Scott (3) was accepted by Henderson (1). Small quantities of 0.8 per cent saline were injected into the muscle mass under measurable increments of pressure. The muscle accepts such injections at reproducible pressures which in the normal human adult varies from 60–90 mm. H_2O. The increment of pressure, resulting from the ability of the muscle to accept injections of saline at reproducible levels, Henderson termed intramuscular pressure (2). A considerable literature has demonstrated the validity of the measurement of intratissue pressure by forcing minute amounts of saline into the tissue (3, 4, 5). These pressures vary significantly with the physiologic and pathologic states of the muscular and circulatory systems. Henderson has shown that intramuscular pressure falls to low levels after anesthesia and surgical procedures (6) and should be low in shock (1). We have verified and extended his observations in postoperative depression and in shock-like states associated with hemorrhage, surgical procedures under anesthesia, and in acute infections (7, 8, 9).

In Henderson's concept, intramuscular pressure is a measurement of muscle tonus, and muscle tonus is measurable during life as intramuscular pressure. Henderson used the term tonus according to the definition of Johannes Müller (19); *as a slight contractile tension characteristic of normal skeletal muscle when at rest.* This, Müller attributed to the influence continually exerted upon the muscle by the nerve centers in the brain. Such rhythmic activity of discrete muscle bundles within the muscle mass should be demonstrable by the detection of electrical action currents. Dr. L. F. Nims in the neuro-anatomy laboratory at Yale University, using a cathode ray oscillograph, demonstrated the presence of rhythmic action currents in the resting biceps brachii muscle of the human being. Thus Müller's contractile tension in the muscle at rest is demonstrated to be related to activity of discrete muscle bundles within the muscle mass. As Henderson and others have shown (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15) this

tension is measurable at reproducible increments by the injection of minute
amounts of saline into the muscle, as intramuscular pressure. It increases as
the muscle performs a definite amount of work, and decreases to a definite resting
level as the muscle mass relaxes (11).   The measurement of intratissue pressure
in the muscle during life is a measurement of muscle tonus.

Krogh (13) showed that individual muscle fiber groups are under different
states of contraction and relaxation.   The amount of blood filling the capillary
and venule bed is related to the degree of activity of the muscle, and to the
number of muscle bundles at any one moment that are in contraction (1, 11, 13).
During maximum contraction the capillary and venule bed of the muscle is
gorged with blood.   Such increasing degrees of muscle activity should show sum-
mation of the electrical action currents, that have been demonstrated in the mus-
cle at rest.   This phenomenon, too, Henderson demonstrated in his laboratory.
He showed that increasing amounts of summation of electrical activity occurred
with increasing degrees of work, performed by the biceps brachii in human
beings.   The increasing degrees of summation of the action currents with work
is correlated with increasing levels of intramuscular pressure.   Decreasing ac-
tivity as the muscle relaxes is similarly paralleled by a decrease in the increments
of intratissue pressure to the resting level (11).

Krogh's morphologic description of the increasing and decreasing vascularity
within a muscle, related to the degree of activity, and the number of muscle bun-
dles contracting within the muscle mass, is in reality the description of a micro-
scopic venous pump (1, 11).

The evidence at hand now lends strong support for Henderson's postulate of
the existence of a venous pump in the circulation of the human being.   This
pump is the mass of skeletal muscle, including the diaphragm.   The energy for
the pump is derived from the isometric tonic activity of the discrete muscle
bundles when the muscle is at rest, and by active contraction of muscles, in the
performance of work.

The energy of tonic muscle activity as well as that of the contracting muscle
is transferred to the venous column and is reflected in the volume of venous flow
to the heart, and in the effective level of venous pressure.

When muscle tonus, i.e., intramuscular pressure, fails, the effective venous
pressure and venous flow necessary to maintain ventricular filling decline, car-
diac output (volume of flow) decreases, and failure of the peripheral circulation
ensues.   This is the venopressor mechanism.

We too have found that the muscle accepts injections of saline at reproducible
increments which range in the normal human between 60 and 90 mm. H_2O (7,
14) and vary significantly in shock and in shock-like states (7, 8, 9).   It is evident
from our studies that the energy of the tonic tension of muscle fibers, measured
in the muscle mass as intramuscular pressure, can be transferred to the venous
bed and be manifested as venous flow and alterations in venous pressure (7, 8, 9,
14, 15).   Contrariwise can the pressures within the venules which so richly sur-
round muscle fibers be imparted to the mass of muscle as increments of intra-
tissue pressure?   Is the resistance of the muscle mass to the injection of fluid,

viz., intramuscular pressure, a result of the pressure within the venous bed measured as venous pressure?

METHODS FOR THE MEASUREMENT OF INTRAMUSCULAR AND VENOUS PRESSURE. Over one thousand measurements of intramuscular pressure have been made in our various studies (7, 8, 9, 14, 15). Henderson's method (6) was used in about half of our observations, and the instrument devised by Gunther and Henstell (15) for the simultaneous and repeated determination of intramuscular and venous pressure in the remainder. Venous pressure was measured by the direct method when Henderson's instrument was used. The level of the auricle was taken at 5 cm. below the level of the chest in recumbency in both methods.

METHODS OF STUDY. The tourniquet experiment described by Wells et al. (4) was employed, using the biceps brachii muscle. Venous pressure was measured in the ante-cubital vein. Simultaneous measurements of intramuscular and venous pressure were made at different increments of venous pressure up to 58 cm. H_2O.

Bedside observations were made on patients suffering from congestive (right heart) failure wherein venous pressures up to 30 cm. H_2O were encountered. Measurements were taken simultaneously in the ante-cubital vein and in the biceps brachii muscle.

Bedside studies were conducted in three patients suffering from syphilis with tabes dorsalis. Simultaneous measurements were made in the antecubital vein and in the biceps brachii muscle in the upper extremity and in the long saphenous vein and the gastrocnemius muscle of the lower extremity. The tourniquet experiment was repeated in the arm and in the leg at different increments of venous pressure.

Studies were made in the operating room during the course of anesthesia and surgical procedures in which the Trendelenburg position was used during surgery. Intramuscular pressure was measured in the biceps brachii and venous pressure in the antecubital vein. The level of the auricles was taken at chest level during the Trendelenburg position.

Observations were made in human beings and in the frog (Rana pipiens) with the needle in situ, immediately before and after death. In human beings the biceps brachii and antecubital veins were used; in the frog, intramuscular pressure was measured in the gastrocnemius and the adductor magnus muscles of the thigh.

These pressures were studied in the frog before and immediately after clamping the branches of the truncus arteriosus at the base of the heart, and of the dorsal aorta caudal to the iliac arteries, to determine the effect of the loss of venous pressure on intramuscular pressure.

RESULTS OF EXPERIMENTS. The effect of artificially induced high increments of venous pressure on intramuscular pressure was studied in 4 tourniquet experiments. The venous pressures induced up to 58 cm. H_2O maintained for 38 minutes showed no significant effect on intramuscular pressure of the corresponding biceps brachii muscle (fig. 1 a and 1 b). One tourniquet experiment was made in the arm and the leg of a patient with tabes dorsalis. He had low

initial values for both the venous and intramuscular pressure. Venous pressure was 3.6 cm. H_2O in the antecubital vein and 4.0 cm. in the biceps brachii, and 40 mm. H_2O in the gastrocnemius muscle. Increasing the levels of venous pressure to 36 cm. H_2O in the antecubital vein and 19 cm. H_2O in the long saphenous vein did not alter the intramuscular pressure in either the biceps or the gastrocnemius (fig. 2).

Four patients suffering from congestive heart failure were studied. They showed venous pressures from 18 to 30 cm. H_2O. The intramuscular pressure
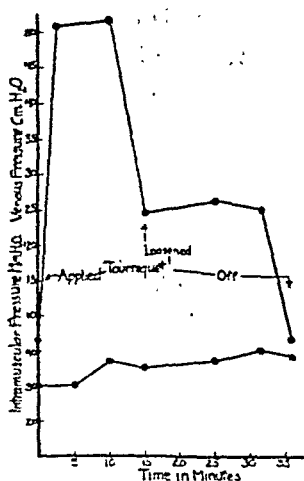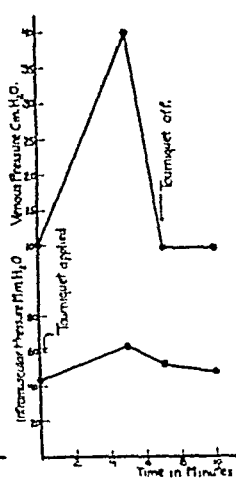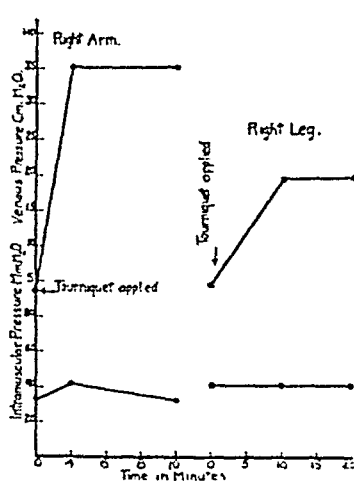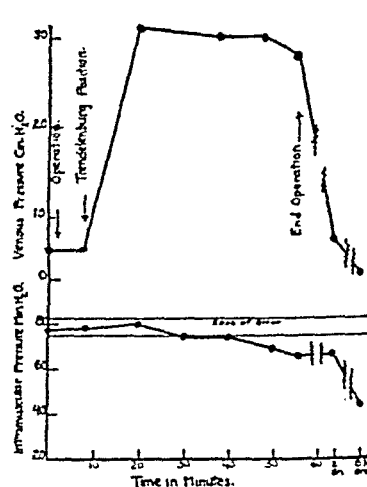
Fig. 1a   Fig. 1b   Fig. 2   Fig. 3

Fig. 1a, 1b. Showing the effect of high levels of venous pressure on intramuscular pressure in the biceps brachii in the human.

Fig. 2. Showing the effect of high levels of venous pressure on the intramuscular pressure in the biceps brachii and the gastrocnemius muscles in the human, suffering from syphilis with tabes dorsalis.

The initial values for intramuscular and venous pressures are below the normal range. The patient did not show signs of peripheral circulatory inadequacy.

Fig. 3. Showing the effect of high levels of venous pressure, induced by gravity, in the Trendelenburg position, during the course of operation under anesthesia.

The high increments of venous pressure did not affect the preoperative level of intramuscular pressure. The usual drop in intramuscular pressure occurred after 40 minutes of surgery despite the maintained high venous pressure. This drop preceded the fall in venous pressure which occurred later. The entire course of events is identical to those which occur in other patients during anesthesia and operation, in normal recumbency and in whom venous pressures were normal at the outset and during the initial period of operation.

values were well within the normal range of 60–90 mm. H_2O observed in persons without congestive heart failure (table 1).

Two patients undergoing pelvic operations in the Trendelenburg position were observed throughout the course of surgery. When placed in this position, the head is considerably lower than the feet and the forces of gravity greatly increase the physiological values of the venous pressure. Venous pressure to 27 and 31 cm. H_2O plus or minus 3 mm. H_2O were recorded. No significant alterations of intramuscular pressure were observed before or after the venous pressure was

increased to these high levels by the effect of gravity (fig. 3 a, 3 b). The Trendelenburg position and the high increment of venous pressure was maintained for from 40–50 minutes during the operation. The high level of the venous pressures in the Trendelenburg position did not prevent the usually observed fall of

TABLE 1

Intramuscular and venous pressures in congestive heart failure

PATIENT	TIME	INTRA-MUSCULAR PRESSURE	VENOUS PRESSURE	BLOOD PRESSURE	REMARKS
		mm. H₂O	*cm. H₂O*	*mm. Hg*	
A10		66	20		
A10a		83	18		Rheumatic heart disease. Auricular fibrillation. Ether time 37 seconds. Vital capacity 1500 cc.
A10b		55	20		Pericardial effusion
A41		64	25		Pericardial effusion
		65	26		
		68	30		Patient excited. Pericardial thoracentesis was attempted
A7		95	11		Before anesthesia
	10 min.	95	11		Before surgery and after inhalational anesthesia
	40 min.	85	27		Trendelenburg position; 40 min. after onset of anesthesia and 30 min. after onset of surgery. Intramuscular pressure still within range of error of the method, but falling
	120 min.	38	4		Later it reached a low level
B7		78	8.0	140/86	Before surgery and anesthesia
	9 min.	80	8.0		Surgery
	19 min.	83	31.0	160/80	Trendelenburg position
	34 min.		31 ± 3		Rapid respiratory fluctuation
	37 min.	75	28		
	40 min.	76	30	170/80	
	48 min.	68	28		
	58 min.	66	28	160/80	Operation ended
	8 hrs. and 43 min.	43	3.6		Post-operative depression

intramuscular pressure which likewise occurs after 40–50 minutes of continuous anesthesia and surgery in the ordinary recumbent position. As has been previously observed, the fall in intramuscular pressure preceded the drop in venous pressure. This phenomenon was recorded while the venous pressure was still at a very high level.

Clamping the branches of the truncus arteriosus at the base of the heart of the frog (Rana pipiens) was followed by a cessation of the heart beat which returned after the clamp was removed. With the arterial outflow clamped and the heart at rest, no significant alterations in intramuscular pressure occurred in the gastrocnemius or adductor magnus muscles (table 2). There was little change at 11 hours, but at 24 hours the intramuscular pressure had risen to double the previous value (table 2).

TABLE 2

Showing the effect on intramuscular pressure in the gastrocnemius of the frog (Rana pipiens) of:
1. Loss of venous pressure by blocking the common iliac artery. 2. Loss of venous pressure by blocking the outflow of the heart. 3. Effect of death, viz., cessation of the heart beat, immediately, 11 hours later and 24 hours later. 4. Effect of death in the human, immediately after shock and peripheral circulatory collapse (loss of venous pressure). 5. Effect of death, 24 hours later, in the human (loss of venous pressure)

	INTRAMUSCULAR PRESSURE	REMARKS
	mm. H₂O	
R. P.*	36	Before and after pithing
	33	Dorsal aorta clamped caudal to the iliacs
	28	Considerable hemorrhage. Before
	26	Branches of truncus arteriosus clamped. Heart stopped beating
	26	Clamps released. Heart beating
	26	Branches of truncus arteriosus clamped. Heart stopped beating
	26	Apparent death. Heart not beating
	29	Heart stopped beating 11 hours
	66	24 hours after heart ceased to beat
A57†	25	5 minutes before death, patient in shock and peripheral circulatory collapse
	25	15 minutes after death. No heart beat
M. C.†	60	Before death. Congestive heart failure
	125	24 hours after death

* Gastrocnemius muscle used in the frog (Rana pipiens).
† Biceps brachii muscle used in the human.

Immediately after death in the human being, a cessation of the heart beat in the frog, the intramuscular pressure did not drop. The levels immediately before and immediately after death were identical (table 2).

The lowest levels to which intramuscular pressure fell before death in the frog or in the human patient was 18 mm. H₂O (10).

DISCUSSION. Our findings verified those of Wells et al. (4) who also were unable to change the level of intramuscular pressure in the biceps brachii by raising venous pressure to high levels with a tourniquet. However, when the tourniquet was placed on the leg and the intramuscular pressure measured in the soleus and

anterior tibial muscles, they found that venous pressures above 20 cm. H_2O increased decidedly the intramuscular pressure in these muscles.

The difference in results obtained in the same experiment on the biceps brachii in the arm, and the soleus and anterior tibial muscles in the leg are explainable on an anatomical basis. The latter muscles possess a very tight fascial covering. The biceps brachii and the gastrocnemius muscles on the other hand do not possess a tight fascia. Thus, beyond certain increments of pressure, the dynamics of a closed, relatively inelastic space, comes into play in muscles covered by tight fascia. The fascia of these anti-gravity muscles serve a useful purpose in the support of the long column of venous blood in the extremities in erect posture (4).

It is an interesting coincidence that up to 20 mm. H_2O venous pressure, there was no significant change in intramuscular pressure even in these tightly covered muscles. Twenty centimeters of water venous pressure is commonly encountered clinically in congestive heart failure. We report herein observations on patients with heart failure with venous pressures up to 30 cm. H_2O without significant alterations in intramuscular pressure (table 1). We feel that muscles that do not possess a tightly investing fascia, give a more accurate reflection of intratissue pressure, in relation to the venules of the venous bed which encompass its fibers, in physiologic and pathologic states, than do the muscles with a tight fascia whose intratissue pressure is subject to the dynamics of increments within a closed space.

We have shown that the energy of the muscles, measured as intramuscular pressure, is transferable to the venous bed in terms of an increase in venous pressure (8, 9, 14). Contrariwise, it is apparent from these experiments in which venous pressure was raised to high levels through the use of the tourniquet, through gravity, and with venoconstrictor drugs (9), that the energy of the venous bed, expressed as an increased level of venous pressure, is not transferable to the muscle mass in terms of intramuscular pressure. In none of these experiments did the intramuscular pressure significantly change in the face of high levels of venous pressure. Even more significant, in our opinion, was the fall in intramuscular pressure that occurred after 40–50 minutes of anesthesia and surgery, in the presence of the high level of venous pressure, which had been maintained for 38 minutes through the forces of gravity. The drop in intramuscular pressure in the presence of a high venous pressure was in no way different from that previously reported by the authors (14) after 40–50 minutes of anesthesia and surgery in the recumbent position, wherein the venous pressure was within normal limits at the beginning of the operation. In a similar fashion, in both circumstances, the initial decrease in intramuscular pressure preceded the drop in venous pressure by 40–50 minutes.

Sympathomimetic drugs which have a venoconstrictor action as well as a pressor action increase venous pressure for from 20 to 40 minutes, but in no way change the level of intramuscular pressure (14, 17). We have indicated that a compensatory mechanism exists akin to the vasoconstriction which increases peripheral resistance to maintain systolic blood pressure which through venoconstriction, alone, sustains venous pressure during surgical procedures for pe-

riods of from 20 to 40 minutes after intramuscular pressure has failed (9, 14). However, if the level of intramuscular pressure continues to remain low beyond this period of time the mechanism fails and venous pressure declines (7, 8, 9, 10, 14).

A total loss of venous pressure, such as occurs after death in the patient and after clamping of the arterial outflow from the heart of the frog, with stoppage of the heart, did not alter the level of intramuscular pressure that existed before the clamping experiment, and before death in the patient or for eleven hours after cessation of the heart beat in the frog (table 2).

We did not encounter intramuscular pressures below 18 mm. H_2O immediately after death in any patient (10) or in the frog. This is the lowest level to which intramuscular pressure falls in postoperative depression, in shock, and in shock-like states in the human being.

Eighteen millimeter H_2O pressure must be the resiliance pressure of the mass of the muscle itself, for as rigor mortis appears the resistance of the muscle mass to the injection of fluid becomes very high. Intramuscular pressures above 18 mm. H_2O during life probably represent the energy of the muscles that is effective in the dynamics of peripheral circulation.

The evidence is strong that intramuscular pressure is a measurement of a quality of the living muscle called tonus. But at death the value of this measurement ceases, inasmuch as tonus disappears with death (2), but the resistance of the muscle to injection of fluid does not disappear with death.

Clinicians have long been aware of the relaxed state of the muscles of a patient suffering from syphilis with tabes dorsalis. Physiologists explained these phenomena in terms of loss of muscle tonus or as hypotonia (16). We have observed low values for intramuscular pressure in tabetic patients. As shown in figure 1, in such a patient, not only was the intramuscular pressure in the biceps brachii and the gastrocnemius muscles lower than is commonly found in the normal healthy individual, but the venous pressure measured in the antecubital vein was likewise lower than was normally found.

We have never observed a low venous pressure with a normal intramuscular pressure nor have we ever observed a low venous pressure without a low level for intramuscular pressure. We have followed patients before and during the development of postoperative depression and shock and into shock-like states with peripheral circulatory collapse and have never observed venous pressure to fall first, and intramuscular pressure later. Contrariwise, after the first 40–50 minutes of anesthesia and surgery, intramuscular pressure fails before the venous pressure drops. Thus, after intramuscular pressure had been depressed for a period upward of 40 minutes, the venous pressure always declined. When a patient was seen for the first time and found to be in shock with peripheral circulatory failure, an initial low intramuscular pressure was always accompanied by a low venous pressure. However, when we had the opportunity to study a patient during the development of shock and peripheral collapse, we found that intramuscular pressure failed first, and the venous pressure later.

The low level of intramuscular pressure seen in peripheral collapse, can be restored to the normal level by the intravenous administration of 10 cc. of a 20

per cent solution of pyridine-b-carboxylic-acid diethylamide[5]. Such a restoration is always accompanied by a simultaneous increase in venous pressure. Similarly, after the inhalation of carbon dioxide diluted in air in the normal (18) and after over-ventilation tetany in the normal, increases in intramuscular and venous pressures were always observed to occur simultaneously (14).

In our experiments and observations, all that could be noted was a, increasing venous pressure by means of a tourniquet, by gravity or by sympathomimetic drugs did not increase intramuscular pressure; b, a high level of venous pressure did not prevent the fall of intramuscular pressure (after 40–50 min. of surgery and anesthesia) which in turn preceded the fall of venous pressure; c, in the hypotonia of tabes dorsalis both intramuscular and venous pressures were found to be at low levels; d, a simultaneous increase of intramuscular and venous pressures always occurred after any agent that increased intramuscular pressure. When clinical improvement in the peripheral circulation occurred with a rise in venous pressure, evidence could not be demonstrated that intramuscular pressure increased before the venous pressure, analogous to the observation that the muscular phenomenon preceded the venous phenomenon when failure in the peripheral circulation occurred.

SUMMARY. Increasing the venous pressure to high increments by use of the tourniquet did not increase the level of intramuscular pressure in the human being.

Increasing the level of venous pressure by gravity in the Trendelenburg position for periods up to 50 minutes did not increase intramuscular pressure in the patient, nor did the maintained high level of venous pressure prevent a fall of intramuscular pressure which occurred after 40–50 minutes of surgical procedure under anesthesia, which in turn preceded the fall in venous pressure.

Sudden clamping of the arterial circulation and at the truncus arteriosus and in the dorsal aorta in the frog did not lower intramuscular pressure in the gastrocnemius or adductor magnus muscles.

A loss of venous pressure after death in the human patient, and after cessation of the heart beat in the frog did not immediately alter the previously existing level of intramuscular pressure, nor did it change in the frog 11 hours after cessation of the heart beat.

Eighteen millimeters H_2O is the lowest level of intramuscular pressure obtained in human beings or in the frog immediately after death; this is the level to which intramuscular pressure falls before death in the shock state in patients, when peripheral collapse has ensued.

The resistance of the muscle mass to the injection of fluid (intramuscular pressure) increases after death and becomes high with the appearance of rigor mortis.

CONCLUSIONS

1. The resistance of the muscle mass to injections of saline at reproducible levels is a valid measurement of tissue pressure but only during life is it a measurement of the property called tonus.

[5] Coramine brand of Nikethamide, Ciba.

2. Intramuscular pressure increments above 18 mm. H_2O represent intratissue energy that is transferable to the venous bed. This transfer of energy can be measured in terms of increments of venous pressure.

3. Venous pressure on the other hand is not a determinant of intramuscular pressure, and the energy of the venous bed, measured as venous pressure, is not transferable to the muscle mass, measured as intramuscular pressure.

REFERENCES

(1) HENDERSON, Y. Adventures in respiration. Baltimore. The Williams & Wilkins Co., 1936.
(2) HENDERSON, Y., A. W. OUGHTERSON, C. A. GREENBERG AND C. P. SEARLE. This Journal 114: 261, 1935.
(3) KERR, J. D. O. AND L. D. W. SCOTT. Brit. Med. J. 2: 758, 1936.
(4) WELLS, H. S., J. B. YOUMANS AND D. G. MILLER. J. Clin. Investigation 17: 489, 1938.
(5) HILLEBRANDT, F. A., E. J. KRIGLER AND L. E. A. KELSO. This Journal 126: 247, 1939.
(6) HENDERSON, Y., A. W. OUGHTERSON, C. A. GREENBERG AND C. P. SEARLE. This Journal 114: 261, 1936.
(7) GUNTHER, L., H. ENGELBERG AND L. STRAUSS. Am. J. Med. Sci. 20: 266, 1942.
(8) GUNTHER, L., H. ENGELBERG AND L. STRAUSS. Am. J. Med. Sci. 20: 271, 1942.
(9) GUNTHER, L., H. H. HENSTELL, L. STRAUSS AND H. ENGELBERG. Am. J. Med. Sci. 204: 394, 1942.
(10) GUNTHER, L. U. S. Naval Bull. 41: 414, March, 1943.
(11) HENDERSON, Y. Science 95: 539, 1942.
(12) ADRIAN, E. D. AND D. W. BRONK. J. Physiol. 67: 119, 1929.
HOEFER, P. F. A. The diseases of the basal ganglia Proc. Assoc. Nerv. and Ment. Dis. The Williams & Wilkins Co. 1: 502, 1942.
(13) KROGH, A. The anatomy and physiology of capillaries. Yale Univ. Press, 1929.
(14) GUNTHER, L., L. STRAUSS, H. H. HENSTELL AND H. ENGELBERG. Am. J. Med. Sci. 204: 387, 1942.
(15) GUNTHER, L. AND H. H. HENSTELL. J. Lab. and Clin. Med. 27: 1339, 1942.
(16) WRIGHT, S. Applied physiology. Oxford Univ. Press., New York, 1942.
(17) NATHANSON, M. H., H. ENGELBERG AND J. HERSH. Am. Heart J. 24: 153, 1942.
IGLAUER, A. AND M. D. ALTSCHULE. J. Clin. Investigation 19: 503, 1940.
(18) GUNTHER, L. AND H. BLOND. Am. J. Med. Sci. 193: 525, 1937.
(19) MÜLLER, J. Quoted by J. F. FULTON. Muscular contraction. 1926, Williams & Wilkins Co., Baltimore, p. 383.

BRAIN METABOLISM DURING ELECTRONARCOSIS[1]

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In mammals a state resembling narcosis has been obtained by the application
of electrical currents to the brain through electrodes located on the outside of
the head. This state, electronarcosis (en.), can be produced by currents with
very different properties; unidirectional pulse current (Leduc, 1902), pulse cur-
rent with alternating direction of the pulses (Nagelschmidt, 1912), 50 cycle
sinusoidal alternating current (van Harreveld and Kok, 1934). It was recently
shown in this laboratory that the electronarcotic effect is due to the stimulative
action of the current applied (van Harreveld, Plesset and Wiersma, 1942). This
fact indicates that the mechanism of en. differs from that of chemical narcosis,
even though the symptoms of the two states may resemble each other very
closely.

Alexander and Cserna (1913) found that ether narcosis causes a severe de-
pression of the brain metabolism. Administration of morphine or magnesium
sulfate also decreased the oxygen consumption of the brain. This was con-
firmed by Gayda (1914). Because of the differences in mechanism of chemical
and electronarcosis it seemed of interest to determine the brain metabolism
during en.

METHODS AND RESULTS. The metabolism of the brain before, during and
after en. was determined by measuring the decrease in oxygen content of the
blood during its passage through the central nervous tissue (A-V difference),
and by measuring the amount of blood flowing through the brain. For techni-
cal reasons it was found impractical to make both of these determinations simul-
taneously; the blood flow experiments require considerable preparation, making
chemical narcosis immediately before the experiment necessary, and since this
interferes with the oxygen uptake of the brain, the changes in A-V difference
were determined in experiments in which no general narcotic was used.

En. was given as described before (van Harreveld, Plesset and Wiersma, 1942).

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[2] Hixon Fellow.

Two electrodes were placed on the temples directly behind the eyes. En. was initiated with 250–300 ma. 60 cycle alternating current. After 30 sec. the current was decreased to a level where respiration returned. With this current the animals could be kept in en. for any time desired.

Dogs were used in all the experiments.

1. *Determination of blood flow in the brain and its relation to blood pressure.* Rein's method of blood flow determination, in which heating electrodes and thermocouples are placed directly in contact with the artery, could not be used in the present experiments since the initiation of en. is characterized by strong convulsions which would result in movements of the electrodes with respect to the vessels. Therefore the combined blood streams of the central ends of the severed carotids were led through flexible tubes to a flow meter placed outside the body and then through a heating coil, back to the peripheral ends of these arteries. Before re-entering the body the blood temperature was measured, and the temperature of the water round the coil was adjusted as to heat the blood to about 38°.

The flow meter used was built on the Rein principle and consisted of a thick walled lucite tube (5 mm. inner diameter) through which the blood stream passed. In the middle of the tube the blood passed between two heating electrodes. These electrodes were 2.5 mm. apart and their shape, size and placement in the canal were such that the blood stream could be heated uniformly by a 60 cycle heating current passing through the blood. The power used was 2 to 3 watts. The temperature difference of the blood before and after passing between the heating electrodes was measured with two thermocouples and a galvanometer. The flow meter was calibrated with defibrinated ox blood. A practically linear relation was found between the blood flow and the reciprocal of the galvanometer deflection per watt of heating power.

Under ether narcosis the thyroid and external carotid arteries were ligated. In some experiments the vertebral arteries were left untouched; in most they were clamped off. In the latter cases care was taken to supply the brain with blood throughout the manipulations, by connecting first one carotid with the flow meter and to connect the other only after the circulation through the first had been re-established. Blood pressure was recorded from the femoral artery. Fastusol B.B.A., 50 mgm./kgm. body weight (Modell, 1939) and heparin[3], 5 mgm./kgm. body weight were used as anticoagulants.

a. *Effect on blood pressure* (fig. 1, broken line). The variations in blood pressure before en. can usually be traced to stimulations of the animal during this period. Immediately after applying the current, a drop in blood pressure occurs. The extent of this drop is rather variable. After a few seconds, however, the pressure rises above the prenarcotic value. This rise usually lasts for several minutes after the initial high en. current has been reduced. The blood pressure eventually falls off to about the level found during the prenarcotic period. These changes are in complete agreement with the observations made by Bikeles and Zbyszewski (1920), Ivy and Barry (1932), Roos and Koopmans

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[3] The heparin was kindly supplied by Hoffman-La Roche, Inc.

(1934) and by van Harreveld and Kok (1934). After the termination of en. a sudden drop in blood pressure is often observed.

b. *Effect on blood flow* (fig. 1, solid line). The data for the calculation of the blood flow (deflection of the galvanometer, voltage and amperage of the heating current) were recorded once a minute. In all experiments the blood flow had a tendency to decrease slowly and continually during en. as well as during the control periods before and after current application. This decline is not caused by changes in the blood pressure as in many cases the mean pressure does not change materially during the entire experiment, except during the short period immediately after the onset of en. Immediately after the start of en. large changes in blood flow occur which parallel the blood pressure variations. During the first seconds of current application, the blood flow drops. This is followed by a sharp rise which often raises the blood flow for a few minutes high above the prenarcotic value. In the next few minutes the flow drops again
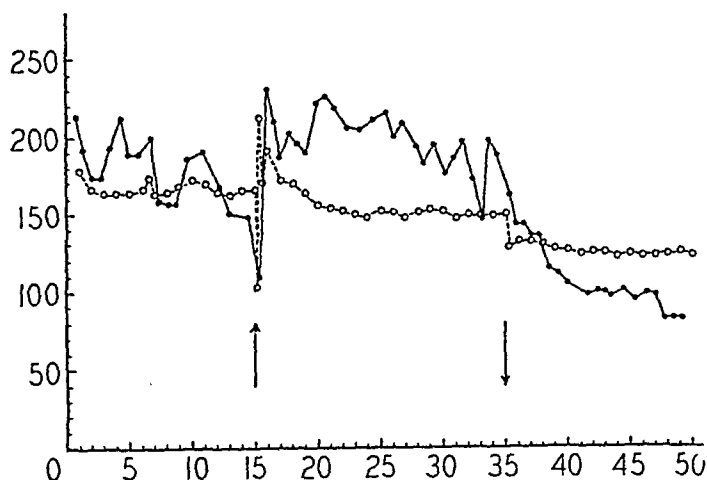
Fig. 1. Blood flow (solid line) and blood pressure (broken line) during a blood flow experiment. On the abscissa is plotted the time in minutes, on the ordinate the blood flow in centimeters per minute and the blood pressure in millimeters Hg. At the arrow pointing up electronarcosis is started, at the arrow pointing down the current is cut.

somewhat. From then on the blood flow continues to decline slowly during the en. period. In this way the blood flow, during the main part of en., is always larger than would be expected from its prenarcotic slope. When the en. current is cut, the flow often drops sharply. A good demonstration of this phenomenon can be seen in figure 1. In this experiment the blood pressure also shows a slight drop at the end of en. The drop in blood flow, however, continues long after the blood pressure has reached its new lower level.

It thus seems that en. raises the level of blood flow without interrupting the continuous decline observed during the control periods before and after en. There is a considerable variation in the rapidity of the decline in different experiments; sometimes the blood flow after 20–30 min. of en. is still well above its prenarcotic level. In other experiments it dropped considerably below this level notwithstanding the rise caused by en.

In some of the control experiments in which the animal was prepared as usual

but in which no en. was given, the same gradual decline of the blood flow was found without, of course, the increase due to en. In other controls, however, the blood flow dropped only for about the first 30 min. but then leveled off. The decline is not a specific effect due to the administration of fastusol since a drop of similar magnitude was found when heparin was used as anticoagulant.

In the experiment given in figure 1, the vertebral arteries were clamped off. A similar picture is obtained when the vertebral arteries are left intact. In the latter experiments the total amount of flow measured through the internal carotids is smaller.

2. *Determination of A-V differences.* On the day before the experiment a $\frac{1}{2}$ inch hole was trephined in the skull over the sagittal sinus. This was performed under nembutal-ether narcosis and with aseptic precautions. Into this hole a threaded piece of brass tubing $\frac{1}{2}$ inch high and $\frac{1}{2}$ inch in diameter was screwed. The spinal cord was transected in the lower thoracic region to make possible recording of the blood pressure from the femoral artery during the actual experiment without the use of a general anesthetic.

The next day the dura, at the bottom of the tube, and the surrounding skin were anesthetized with novocain, and a femoral artery was prepared and connected with a recording manometer. The manometer connections and the cannula were filled with 5 per cent fastusol solution and 50 mgm. of the dye per kgm. bodyweight was injected intravenously.

The venous samples were taken in the following manner: the brass tube was filled with thin mineral oil, then the sinus was punctured and the blood accumulating under the oil was sucked up in a syringe partly filled with oil. The tube was then closely packed with cotton until the next sampling. Arterial samples were obtained from the intact femoral artery. The samples (5 cc.) were transferred under oil to tubes cooled by ice water until the time of the oxygen determination. It was found that blood can be kept for several hours under these conditions without significant changes in the oxygen content. The oxygen content of the samples was determined by the method of van Slyke and Neill (1924).

In most experiments 6 sets of venous and arterial samples were drawn, namely, two controls, about 20 and 5 min. before the start of en.; two sets during en. 10 to 15 and 25 to 30 min. after the start; and two more controls 10 to 15 and 25 to 35 min. after the end of en. The first sample during en. cannot be taken earlier than about 10 min. after the start because, as has been shown above, rapid and large changes in blood flow occur during the initial phases of en. Besides there is a complete respiratory arrest during the first minute of en.

In table 1 the oxygen A-V differences of 7 experiments are given. The overall values show that the A-V differences increase notably during the experiment. This tendency is present in the period before, during and after en., as a comparison of the two values obtained during each of these periods shows. The effect of en. is to depress the A-V differences without interrupting the slow increase. Thus the beginning of en. causes a drop of the A-V differences, whereas a notable increase of this value is observed after the end of en. The individual experiments show considerable deviations from this average. Especially it should be

noted that in some experiments the A-V differences during en. instead of being smaller are larger than before current application.

DISCUSSION. It is likely that the slow decline in blood flow observed is due to a number of factors among which the cessation of ether narcosis, the use of

TABLE 1

A-V differences during electronarcosis experiments

Each observation consists of three figures. The upper one indicates the time in minutes before the start of en. (columns 1 and 2), after the beginning of en. (columns 3 and 4), and after cutting the current (columns 5 and 6). The middle of the three figures is the A-V difference in volume per cent at these moments, and the lower figure is the blood pressure in millimeters Hg at the moment the sample was taken.

NUMBER	BEFORE ELECTRONARCOSIS		DURING ELECTRONARCOSIS		AFTER ELECTRONARCOSIS	
1	20′ 13.3 % 103 mm.	5′ 13.0 % 117 mm.	15′ 8.8 % 95 mm.	30′ 11.5 % 102 mm.	15′ 16.1 % 100 mm.	30′ 16.6 % 86 mm.
2	22′ 9.8 % 99 mm.	7′ 9.8 % 98 mm.	11′ 11.5 % 85 mm.	28′ 13.5 % 98 mm.	10′ 13.6 % 79 mm.	
3	18′ 14.8 % 134 mm.	6′ 16.3 % 123 mm.	10′ 17.9 % 123 mm.	26′ 19.6 % 139 mm.	12′ 19.1 % 124 mm.	25′ 19.9 % 100 mm.
4	21′ 11.8 %	5′ 12.8 %	12′ 12.8 %	28′ 12.3 %		35′ 17.2 %
5	20′ 13.3 % 128 mm.	5′ 15.1 % 132 mm.	15′ 13.3 % 159 mm.	30′ 11.8 % 148 mm.	16′ 13.5 % 105 mm.	35′ 12.3 % 90 mm.
6	19′ 13.4 % 96 mm.	4′ 15.1 % 123 mm.	16′ 12.2 % 98 mm.	31′ 12.9 % 109 mm.	15′ 15.3 % 100 mm.	30′ 15.1 % 106 mm.
7	20′ 12.6 % 80 mm.	5′ 10.5 % 118 mm.	10′ 9.3 % 108 mm.	25′ 11.8 % 124 mm.	8′ 12.6 % 92 mm.	28′ 13.5 % 94 mm.
Average A-V differences..	12.7 %	13.2 %	12.3 %	13.3 %	15.0 %	15.8 %

an anticoagulant, and perhaps the application of the en. current have to be considered.

The administration of ether, necessary for the preparation of the animal for the blood flow experiments, was stopped shortly before the beginning of the blood flow recording. Alexander and Cserna (1913) and Gayda (1914) found

that the blood flow is decreased during chemical narcosis. This would exclude the possibility of explaining the slow decline of the blood flow observed in the present experiments by the cessation of ether narcosis. However, Finesinger and Cobb (1935) saw that the pial vessels are dilated during ether narcosis. These vessels contracted again when narcosis was stopped. Such a contraction, if present throughout the brain, would explain the decline of the blood flow during the first part of the experiment.

There are indications that a brain edema develops under the experimental conditions, for at the end of the blood flow experiments brain material was found to protrude through a hole made in the skull. The development of this brain edema may explain the continued slow decline in blood flow. Since Jacobi and Magnus (1925) observed the development of brain edema during en., it is possible that the application of the en. current at least facilitates the development of the edema. On the other hand, the same slow decline of blood flow throughout the experiment has been found in some of the controls. It is possible that the use of an anticoagulant has caused the edema in these cases. Since the same amount of fastusol has been injected in the blood flow and in the A-V difference experiments, the two series of experiments are comparable in this respect.

A parallel between the blood pressure and blood flow exists during the earlier stages of en. which is indicative of the close dependence of these two values. However, the mean blood pressure is not the only factor influencing the rate of flow. It was found that the blood flow level is raised for the duration of en. This is true even if the blood pressure during en. is lower than before (see fig. 1). The stimulating effect of en. on the blood flow may be due to a dilating effect on the brain vessels. This is supported by an observation of Jacobi and Magnus (1925), who found that the pial vessels during en. are markedly dilated.

Most of the A-V differences found in the present experiments on non-narcotized animals are between 12 and 16 vol. per cent. In some experiments in which A-V differences were determined shortly after stopping ether narcosis, much lower values, between 4 and 8 vol. per cent, were found. These figures are in complete agreement with Alexander and Cserna's values under the same circumstances.

The trend in the changes of the A-V differences (table 1) and of the blood flow (fig. 1) are in opposite directions. Thus, whereas en. depresses the A-V differences, it raises the level of the blood flow. Also, whereas the A-V differences gradually increase during the periods before, during and after en., the blood flow gradually declines. Thus it appears that all changes in the A-V differences found in these experiments are mainly reflections of the changes in the rate of blood flow. It is true that in some experiments (expts. 2 and 3, table 1) the A-V differences 10 to 15 min. after the start of en. were higher than before, but in these experiments the decline of the blood flow may have been so rapid that the flow has fallen below the prenarcotic value at the time the sample was taken.

Since the changes in A-V difference caused by en. seem to be mainly due to variations in the blood flow, it can be concluded that the changes in brain metabolism produced by en., if any, certainly are not large. The question whether

en. influences the brain metabolism in the same way as ether narcosis must thus be answered negatively, and the present experiments support the conclusion (van Harreveld, Plesset and Wiersma, 1942) that the nature and mechanism of electronarcosis differ widely from those of chemical narcosis.

We are indebted to Dr. M. S. Plesset, Mr. C. H. Ellis and Mr. E. B. Wright for their advice and assistance.

SUMMARY

1. The influence of electronarcosis on the blood flow through the brain has been investigated. Electronarcosis has been found to have a tendency to increase the flow.

2. In other experiments in which no general narcotic was applied, the effect of electronarcosis on the A-V differences of the brain was examined. This effect appeared to be a depression of the A-V differences.

3. A comparison of the changes of the A-V differences before, during and after electronarcosis, with the course of the blood flow during these same periods, showed that the former are mainly reflections of the latter.

4. It therefore can be concluded that electronarcosis does not cause large changes in the brain metabolism and thus that electronarcosis differs greatly from chemical narcosis in its effect on the oxygen consumption of the central nervous system.

REFERENCES

ALEXANDER, F. G. AND S. CSERNA. Biochem. Ztschr. 53: 100, 1913.
BIKELES, G. AND L. ZBYSZEWSKI. Pflüger's Arch. 182: 157, 1920.
FINESINGER, J. E. AND S. COBB. J. Pharmacol. 53: 1, 1935.
GAYDA, T. Arch. di Fisiol. 12: 215, 1914.
HARREVELD, A. VAN AND D. J. KOK. Arch. néerl. Physiol. 19: 24, 1934.
HARREVELD, A. VAN, M. S. PLESSET AND C. A. G. WIERSMA. This Journal 137: 39, 1942.
IVY, A. C. AND F. S. BARRY. This Journal 99: 298, 1932.
JACOBI, W. AND G. MAGNUS. Ztschr. ges. Neurol. Psych. 98: 708, 1925.
LEDUC, S. C. R. Acad. Sci., Paris 135: 199, 1902.
MODELL, W. Science 89: 349, 1939.
NAGELSCHMIDT, F. Berl. Klin. Wchnschr. 1849, 1912.
ROOS, J. AND S. KOOPMANS. Vet. J. 90: 232, 1934.
SLYKE, D. D. VAN AND J. M. NEILL. J. Biol. Chem. 61: 523, 1924.

EFFECT OF ATROPINE ON UTERINE RESPONSES TO HYPOGASTRIC NERVE STIMULATION. ACTION AT THE GANGLIONIC SYNAPSE

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Cocaine, which generally potentiates the peripheral activity of adrenergic nerve fibers, does not augment the uterine responses to hypogastric nerve stimulation in the rabbit and monkey, as it does in the cat and rat (1). These results with cocaine might be interpreted as indicating a predominance of adrenergic elements in the hypogastric nerve of the cat and rat, and of cholinergic (postganglionic) fibers in the same nerve of the rabbit and monkey. It seemed possible that this hypothesis might be tested by studying the effect of atropine, a parasympathetic depressant, on the same uterine responses to hypogastric nerve stimulation.

Reports of previous studies on the effect of atropine on uterine responses to hypogastric nerve stimulation are limited to a few brief but authoritative statements. Röhrig (2) in 1879 observed that in the rabbit atropine did not paralyse the "motor" fibers to the uterus. This was confirmed by Langley and Anderson (3) who reported that they had injected up to 50 mgm. atropine intravenously, presumably in the rabbit. No further details of the experiment were given. Cushny (4) observed that "atropine had no effect on the uterus or on the hypogastric nerve, unless pilocarpine had been injected previously, when atropine restored the organ to its normal condition and prevented any further contractions from pilocarpine". These observations of Cushny have been "repeatedly confirmed" by Sherif (5).

The results of the present investigation have substantiated these earlier findings in regard to small doses of atropine. Large doses, on the other hand, depressed and frequently abolished the excitatory response of the uterus to hypogastric nerve stimulation, in both the rabbit and monkey. This difference, depending upon the dose of atropine given, may well explain why others have not reported any effect with atropine. Neither Röhrig nor Cushny stated the amount of atropine which they used, and although Langley and Anderson injected "up to 50 mgs.", which is admittedly a large dose, it is not easy to evaluate their statement without further experimental data. It is possible that with this large dose a reduction in response might have been unrecognizable by naked-eye observation, for Langley and Anderson did not use any mechanical recording of uterine activity.

In carrying out the experiments with large doses of atropine, we were finally led to suspect that the drug might be acting at the ganglionic synapse, rather than at the neuro-effector junction in the uterus.

METHOD. The experimental set-up for hypogastric nerve stimulation and

the recording of uterine activity were the same as previously reported (1). Seventeen experiments in rabbits and 6 in monkeys (Macaca mulatta) were carried out. The animals were anesthetized with nembutal, 0.03 gram per kgm. of body weight, injected intraperitoneally. Atropine sulphate in 0.25 per cent solution was administered intravenously in doses of 1 mgm. to 25 mgm. These doses are highly toxic but were never lethal.

In order to determine whether the effect of atropine on uterine activity and nerve stimulation varied according to the sexual cycle of the uterine musculature, castration was performed one to three weeks prior to the experiment in seven of the rabbits. Three of these animals were given intraperitoneal injections of theelin (2000 rat units) two days, and again one day, before the experiment. In two other non-castrate rabbits, progynon (2000 rat units intraperitoneally) or pregnancy urine (10 cc. intravenously) was administered a day or two prior to the experiment.

RESULTS. There was no essential difference in the results obtained in the rabbit and in the monkey. In only three experiments was the injection of atropine followed by any excitation of the uterus; in all the others atropine had no direct effect on uterine activity.

Small doses of atropine. The excitation of uterine activity seen characteristically in the rabbit and monkey after hypogastric nerve stimulation, was unaltered in four experiments (nos. 4, 17, 18, 46) following the administration of atropine in small doses under 5 mgm. Increasing the total dosage of atropine to 8–10 mgm. during a 10 minute period, in three of these experiments, caused depression or complete inhibition of the response. In the fourth experiment (no. 4) the amount of atropine was increased up to 15.5 mgm. in a 25 minute period, without any alteration of the response.

Large doses of atropine. In three experiments (nos. 43, 44, 48) an initial dose of 6–10 mgm. was followed immediately by marked reduction or cessation of the response to hypogastric nerve stimulation. Recovery of the response in these animals (all monkeys) did not occur for one to two hours. In the remaining 16 experiments, the initial dose of atropine was 12.5 mgm. in 14 animals, and 25 mgm. in 2 others. The response to nerve stimulation was abolished in three (nos. 1, 10, 11), diminished in ten, and unaffected in three (nos. 9, 5, 60). Where the response was reduced by the initial dose, a second administration of atropine (usually 12.5 mgm. within 5–10 min.) produced a further diminution or complete inhibition (fig. 1). The dose necessary to abolish the response varied greatly and in some animals 50 mgm. were administered before complete inhibition followed. With increase in the strength of the stimulus a small response of the uterus could occasionally be reproduced. In 2 of the 3 experiments where the first dose of atropine had no effect on nerve stimulation, a second dose of 12.5 mgm. inhibited the response in one (no. 9), but not in the other (no. 5).

It is clear from these findings that, despite an occasional "negative" result, a large dose of atropine (usually 10–25 mgm.) depressed or abolished the uterine response to hypogastric nerve stimulation in both the rabbit and monkey. Depression of the response by atropine persisted for periods of $\frac{1}{2}$–2 hours, when

hypogastric nerve stimulation once more produced its customary excitatory effect on uterine activity. The occasional negative result (nos. 5, 60), where even 25 mgm. atropine failed to affect hypogastric nerve stimulation, is puzzling. Perhaps a still larger dose in these experiments might have altered the response. One of these animals was a rabbit to which 10 cc. pregnancy urine had been administered 4 days previously and the uterus was markedly active. The other was a normal monkey.
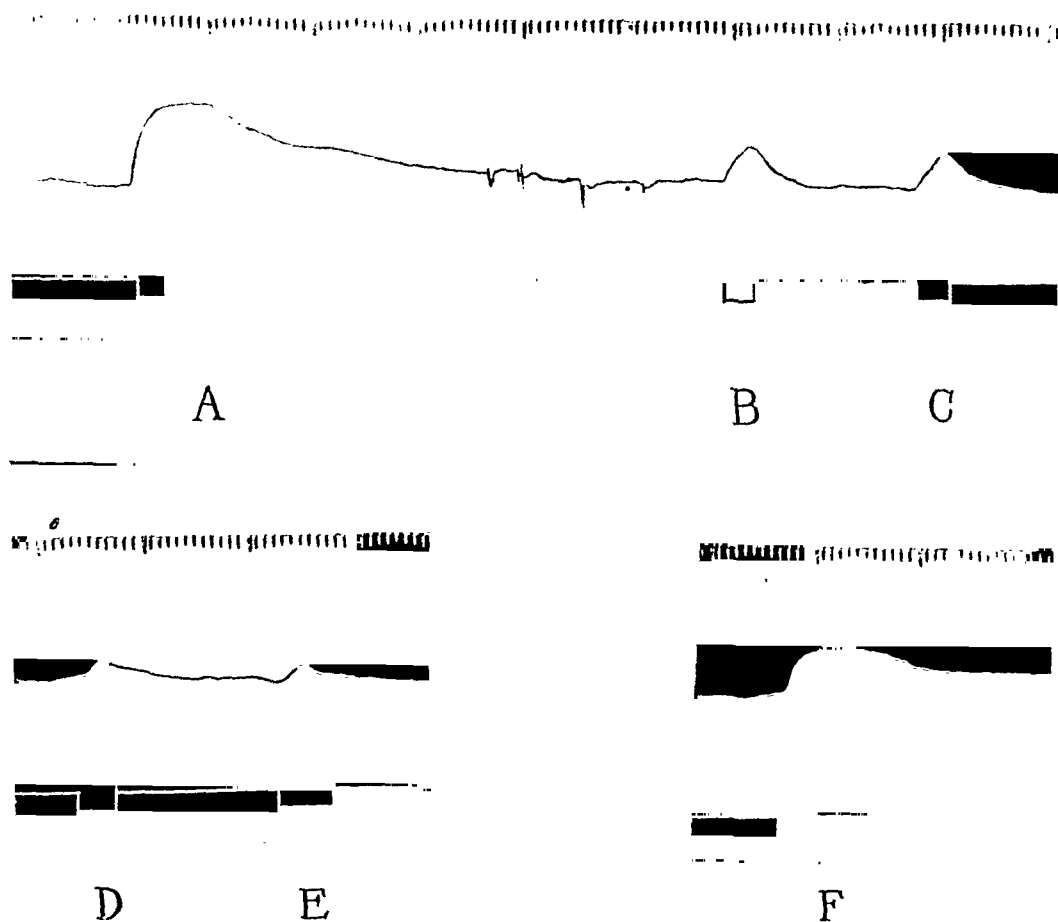
Fig. 1. Effect of large doses of atropine on the uterine response to hypogastric stimulation in the rabbit.

A B C D E F, Stim. hypogastric nerve. Between A and B atropine 12.5 mgm. intravenously. Between C and D 5 min. interval, and atropine 12.5 mgm. intravenously. Between E and F 45 min. interval. Recovery of response.

Time in minutes.

There was no alteration in the uterine response to adrenaline following the administration of atropine.

The results did not reveal any obvious correlation between the effect of atropine on nerve stimulation and the sexual cycle of the uterus. The same depression of the response by atropine was noted in normal, castrate, estrogenized, and pseudo-pregnant rabbits.

In normal monkeys the excitatory phase of the response to hypogastric nerve stimulation is frequently followed by a period of inhibition, during which rhythmic activity in the uterus is reduced to a minimum. Under certain circumstances, such as after the intravenous injection of suitable doses of nicotine, the excitatory phase is abolished but the inhibitory phase persists (6), showing that they are to some degree independent of each other. Following the administration of large doses of atropine the inhibitory phase of hypogastric nerve stimulation clearly persisted in two instances although the excitatory phase had completely disappeared. In the other experiments the inhibitory phase of hypogastric nerve stimulation was not sufficiently prominent to allow interpretation of the results.

DISCUSSION. The dose of atropine required to abolish parasympathetic responses at the neuro-effector junction is small. In the cat 3–6 mgm. is sufficient to paralyse the chorda tympani (7) and even smaller doses have been shown to abolish the effects of vagal stimulation on the heart (8). The fact that much larger doses of atropine (10–25 mgm. and occasionally as much as 50 mgm.) were required to abolish the uterine response to hypogastric nerve stimulation would seem to suggest some action other than that at the termination of cholinergic postganglionic fibers.

There is considerable evidence to show that large doses of atropine act at the sympathetic ganglionic synapse. Langley (7), in studying the depressant action of atropine on salivary secretion found that, after 10 mgm. of atropin had been injected, the sympathetic trunk in the neck no longer could be made to cause a secretion, whilst a secretion could still be obtained from the filaments proceeding from the superior cervical ganglion. Langley did not interpret this experiment further, but others (9, 10) have confirmed the observation. The final proof as to the site of action of the drug in these experiments was given by Feldberg and Vartiainen (11). They perfused the superior cervical ganglion and observed that, although atropine in low concentrations when added to the perfusion had no effect, yet if larger doses, such as 0.1 mgm., were injected into the perfusion, a complete paralysis of response to preganglionic impulses and to acetylcholine was produced. The relatively smaller dose (2 mgm. injected into the general circulation) which Marrazzi (12) found sufficient to depress transmission through the superior cervical ganglion may be attributable to the fact that he used threshold stimulation of the cervical sympathetic trunk and a highly sensitive method of recording the effect.

Further evidence of the action of atropine at sympathetic ganglia was furnished by the following experiment, which was carried out before we became aware of the similar observation by Langley (7) on salivary secretion. The cervical sympathetic nerve in the cat was stimulated below the superior cervical ganglion, resulting in wide dilatation of the pupil and retraction of the nictitating membrane. Atropine was injected intravenously in graduated doses until a total of 12.5 mgm. was reached. At this point stimulation of the cervical sympathetic trunk below the superior cervical ganglion no longer produced any response in the eye, but stimulation *above* the superior cervical ganglion resulted

in immediate dilatation of the pupil and full retraction of the nictitating membrane.

It has been established (3, 4, 6) already that within the hypogastric nerve pathway to the uterine musculature, synaptic connections lie peripheral to the point of stimulation of the nerve, so it is theoretically possible that the effects that have been obtained with atropine are due to a depressant action at the synaptic junctions in such peripherally located ganglia. The large amounts of atropine which were found necessary to produce any effect make this interpretation probable. It is not permissible, therefore, to draw any further conclusions in regard to the nature of the postganglionic fibers within the hypogastric nerve.

The failure of small doses of atropine to depress hypogastric nerve activity cannot be interpreted necessarily as signifying that these nerves in the rabbit and monkey are not predominantly cholinergic, for there are notable examples where the activity of purely cholinergic (postganglionic) fibers is unaffected by the administration of atropine (13).

SUMMARY

1. Small doses of atropine (up to 5 mgm.) had no effect on the uterine response to hypogastric nerve stimulation in the rabbit and monkey.

2. Larger amounts of atropine (usually 10–25 mgm.) depressed and often abolished the responses for periods lasting ½ to 2 hours.

3. The results with the larger doses of atropine are probably due to the depressant action of the drug at the synaptic connections which lie along the hypogastric nerve pathway, distal to the point of stimulation.

4. Further confirmation of the action of atropine at sympathetic ganglia has been obtained by observing its effect on the changes in the pupil and nictitating membrane in response to stimulation of the cervical sympathetic below and above the superior cervical ganglion.

REFERENCES

(1) LABATE, J. S. J. Pharmacol. and Exper. Therap. 72: 370, 1941.
(2) RÖHRIG, A. Virchow's Arch. f. path. Anat. 76: 1, 1879.
(3) LANGLEY, J. N. AND H. K. ANDERSON. J. Physiol. 19: 122, 1895.
(4) CUSHNY, A. R. J. Physiol. 35: 1, 1906.
(5) SHERIF, M. A. F. J. Physiol. 85: 298, 1935.
(6) SHEEHAN, D. AND J. S. LABATE. This Journal 137: 456, 1942.
(7) LANGLEY, J. N. J. Physiol. 1: 96, 1878.
(8) DALE, H. H. J. Pharmacol. and Exper. Therap. 6: 147, 1914.
(9) BRADFORD, J. R. J. Physiol. 8: 86, 1887.
(10) CANNON, W. B. AND McK. CATTELL. This Journal 41: 39, 1916.
(11) FELDBERG, W. AND A. VARTIAINEN. J. Physiol. 83: 103, 1934.
(12) MARRAZZI, A. J. Pharmacol. and Exper. Therap. 65: 18, 1939.
(13) DALE, H. H. AND J. H. GADDUM. J. Physiol. 70: 109, 1930.

RÔLE OF VITAMIN E IN NEUROMUSCULAR ATROPHY AND REGENERATION[1]

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Numerous investigators have observed that diets deficient in vitamin E lead to degeneration, paralysis and weakness of the skeletal muscles in various species of laboratory animals. The evidence concerning the extent to which vitamin E deficiencies are associated with lesions of the nervous system appears to be controversial and inconclusive (1, 2). Likewise, the reports concerning the efficacy of vitamin E therapy in various neuromuscular diseases have been conflicting.

This report is concerned with the rôle of vitamin E in neuromuscular atrophy and regeneration. Studies have been made concerning the effects of vitamin E-deficient diets and excess intakes of vitamin E upon the extent and velocity of denervation atrophy of muscle and subsequent neuromuscular regeneration. The experiments have been carried out on the gastrocnemius muscles and tibial nerves of guinea pigs and adult albino rats. In the former species the course of muscle degeneration resulting from deficient E intakes is extensive and rapid while in the latter the dystrophy follows a more chronic course.

EXPERIMENTAL. The mothers of young rats were placed on a vitamin E-deficient diet (3) ten days after the birth of their litter. The young were weaned at the age of 28 days and reared for 4 months on the deficient diet. The controls were animals of the same age, sex distribution and stock and were reared on an adequate diet. The members of the E-excess group received a daily supplement of 4 mgm. of alpha-tocopherol[2] per os.

Guinea pigs were placed on a standard E-deficient diet ·(4) and 5 mgm. of ascorbic acid per day. The group designated as "E-control" received in addition 3 mgm. of alpha-tocopherol by mouth every other day. The "E-deficient" guinea pigs received the standard E-deficient diet without alpha-tocopherol supplements. The animals in the groups designated as "E-excess" were given the basal diet supplemented with 15 mgm. of alpha-tocopherol on alternate days.

Complete denervation of the gastrocnemius was accomplished by crushing the tibial nerve at its junction with the peroneal between a heavy linen ligature and a brass rod. Such a procedure allowed for complete paralysis of the muscle and preserved good alignment for subsequent reinnervation. In certain experiments where it was desirable to eliminate muscle regeneration a section was removed from the nerve. The corresponding muscle and nerve of the unoperated contralateral limb were utilized as a control.

At indicated times after operation, studies were made as to the strength, weight and creatine concentration in the control and experimental muscles. The muscles of some animals were utilized for histologic examination. The strength of the muscles was determined by measuring the maximal isometric tension which developed in response to volleys of supermaximal stimuli applied to the tibial nerve and directly to the muscle. The techniques employed for stimulation and muscle strength measurements have been described in detail elsewhere (5). These procedures made it possible at any chosen time to determine muscle strength, weight, creatine concentration, and the capacity of the nerve to activate its muscles; and to compare such findings with those on the unoperated contralateral control limb. Previous studies on the rat have indicated that neuromuscular regeneration runs a remarkably constant course when the animals are carefully matched as to age, body weight and sex (5).

RESULTS. The data in tables 1 and 2 indicate that excess intakes of vitamin E had no appreciable effect upon the course of neuromuscular regeneration. Essentially the same extent of recovery of mass and strength occurred in the denervated muscles of guinea pigs and rats which had received excess intakes of E as in the denervated muscles of the control animals. The functional state of regenerating nerve, as judged by its capacity to activate muscle, was not improved by an excess of vitamin E.

The unoperated control muscles of guinea pigs and rats on vitamin E-deficient diets showed evidence of dystrophy. The creatine concentration in such muscles was found to be somewhat lower than that in muscles from control animals reared on an adequate diet. Histologic examination revealed the presence of lesions characteristic of muscle dystrophy. Functional impairment of muscle was evidenced by the finding of a weaker response per unit weight to direct and motor nerve stimulation than in muscles from control animals reared on diets adequate in vitamin E content. Furthermore, the ratio of tension elicited by nerve stimulation to that developed in response to direct muscle stimulation was considerably lower in animals deficient in vitamin E than in animals on adequate intakes. The latter finding indicates a relative ineffectiveness in the capacity of the nerve to activate its muscle and is significant in the light of a report by Telford (6) that a marked reduction occurred in the number of nerve terminals in the degenerated areas of muscles from rats on vitamin E-deficient diets.

The average values for the results of experiments on 65 rats reared on an E-deficient diet together with values for a larger number of control animals on adequate diets are presented in table 1. The results show that a diet so deficient in vitamin E as to allow the development of mild muscle dystrophy did not appreciably retard recovery from peripheral nerve injury. The loss of muscle strength and weight during a 14 day period of complete denervation was somewhat greater than that found in control animals. However, it is to be noted that in the regeneration experiments recovery of muscle mass and strength occurred at a velocity comparable to that observed in the experiments on control animals.

Studies made upon the gastrocnemius muscle of E-deficient guinea pigs 14

TABLE 1

A summary of the average values and standard errors for gastrocnemius muscles of rats on control, E-deficient and E-excess diets

CONDITION	NO. OF ANI-MALS	TIME AFTER LESION	PER CENT WEIGHT LOSS†	TENSION PER GRAM MUSCLE WHEN ACTIVATED THROUGH				RELATIVE STRENGTH OF DENERVATED MUSCLE† WHEN ACTIVATED THROUGH		CREATINE IN MGM. PER 100 GRAMS MUSCLE	
				Nerve		Muscle					
				Exper.*	Control	Exper.*	Control	Nerve	Muscle	Exper.*	Control
		days									
Control....	14	12	30.5 ±1.2			1257	1878		43.8 ±1.8	340	452
E-def.......	14	12	41.0 ±2.4			785	1431		34.0 ±3.0	297	401
Control....	10	14	39.4 ±1.4			965	1700		35.1 ±1.9		
E-def.......	6	14	45.2 ±0.9			846	1419		32.9 ±2.1		
Control....	15	18	33.7 ±1.5	838	1895	1763	2037	21.7 ±2.5	48.2 ±3.0	347	444
E-def.......	12	18	35.1 ±2.0	398	1261	1010	1793	20.7 ±2.5	41.3 ±2.2	326	426
Control....	34	21	38.7 ±2.0	670	2059	1350	1958	22.0 ±2.1	45.8 ±2.5	355	454
E-def.......	13	21	36.7 ±2.3	561	1253	1067	1528	28.5 ±2.1	44.0 ±2.4	348	416
Control....	20	35	27.0 ±1.6	1322	1591	1697	1842	57.4 ±4.0	63.4 ±2.6	395	451
E-def.......	10	35	29.5 ±1.3	909	1403	1146	1644	45.5 ±2.2	56.1 ±2.2	355	422
Control....	9	42	21.0 ±1.7	1580	1820	1707	1878	69.0 ±2.9	70.7 ±2.3		
E-def.......	16	42	20.1 ±1.5	976	1191	1237	1563	61.4 ±2.5	63.4 ±2.0	370	426
Control....	26	28	26.0	1018	1412	1399	1712	48.9	59.4	410	458
E-excess...	22	28	26.9	1084	1466	1450	1735	54.7	61.2	386	455

* Exper. refers to muscles previously subjected to denervation and control to contralateral non-denervated muscles.

† Expressed as per cent of that found in contralateral control.

TABLE 2

A summary of the average values for gastrocnemius muscles of guinea pigs on control, E-deficient and E-excess diets

CONDITION	NO. OF ANIMALS	TIME AFTER LESION	PER CENT WEIGHT LOSS†	TENSION PER GRAM MUSCLE WHEN ACTIVATED THROUGH				RELATIVE STRENGTH OF DENERVATED MUSCLE† WHEN ACTIVATED THROUGH	
				Nerve		Muscle			
				Exper.*	Con-trol	Exper.*	Con-trol	Nerve	Muscle
		days							
Controls..........	17	35	20.8	1007	1643	1237	1834	47.6	54.5
E-excess..........	9	35	24.6	1040	1631	1439	1845	48.2	59.0
E-def.............	9	35	6.2	524	406	737	582	145.2	127.0
Controls	10	14	31.8			669	1778		25.7
E-def.............	6	14	35.5			378	1257		19.4

* Exper. refers to muscles previously subjected to denervation and control to contralateral non-denervated muscle.

† Expressed as per cent of that in contralateral control.

days after denervation by nerve section showed slightly greater losses of strength and weight than was found in similarly treated muscles of control animals on adequate diets (table 2). However, the relatively short duration of the experiment prohibited the development of more than a mild degree of dystrophy in these animals. A study of regeneration in guinea pigs on diets deficient in vitamin E showed that muscles, 35 days after the crushing of their nerves, were appreciably stronger than their unoperated contralateral controls (table 2). This was true for the responses elicited by nerve stimulation as well as by direct activation. Histologic examination showed the presence of dystrophic lesions in both the denervated and control muscles but quantitative estimations of their severity were difficult. The fact that the unoperated contralateral control muscles gave very weak responses to direct and motor nerve stimulation indicated the presence of a severe state of dystrophy in this group of animals. Thus, it would appear as if the effects of a marked E-deficiency were more pronounced on normally innervated muscles than on muscles previously subjected to denervation. It should be noted that Pappenheimer (7) found that nerve section protected the muscles of young suckling rats from the usual effects of E deprivation. Our knowledge concerning the rôle of vitamin E in muscle physiology and the factors that influence the requirements of tissue for the vitamin must be more complete before such phenomena can be satisfactorily explained.

The experiments on different levels of vitamin E intakes show that neuromuscular regneration is little affected by the quantity of vitamin in the diet. If vitamin E is necessary for neuromuscular regeneration, the requirements are so small as to be within the amounts present in an "E-deficient diet" which readily permits the occurrence of lesions characteristic of E-deficiency. These findings on experimental animals suggest the futility of employing large doses of vitamin E in order to promote recovery of muscle and nerve from lesions resulting from causes other than a deficiency of vitamin E itself.

SUMMARY

A comparative study has been made of neuromuscular atrophy and regeneration in the gastrocnemius muscles and tibial nerves of guinea pigs and albino rats on different levels of vitamin E intakes.

The studies included determinations of muscle strength as measured by the response to stimuli applied directly to muscle and to its motor nerve, the degree of atrophy and creatine concentration at various times after unilateral denervation. The muscle and nerve of the contralateral limb served as a control.

The extent and velocity of neuromuscular regeneration in the rat was not significantly affected by the amount of vitamin E in the diet. The intakes ranged from that in E-deficient diets to that in adequate diets supplemented with several times the usual intakes of vitamin E.

Excess intakes of vitamin E exerted no appreciable effect upon the course of neuromuscular regeneration in the guinea pig. The gastrocnemius muscles of E-deficient guinea pigs undergoing regeneration following a unilateral crushing

of the tibial nerve were found to be functionally superior to their contralateral unoperated controls in the tests made 35 days after operation.

REFERENCES

(1) WOLF, A. AND A. M. PAPPENHEIMER. Arch. Neurol. and Psychiat. **48:** 538, 1942.
(2) WOLBACH, S. B. AND O. A. BESSEY. Physiol. Rev. **22:** 223, 1942.
(3) OLCOTT, H. S. J. Nutrition **15:** 221, 1938.
(4) SHIMOTORI, O., G. A. EMERSON AND H. M. EVANS. J. Nutrition **19:** 547, 1940.
(5) HINES, H. M., J. D. THOMSON AND B. LAZERE. This Journal **137:** 527, 1942.
(6) TELFORD, I. R. Anat. Rec. **81:** 171, 1942.
(7) PAPPENHEIMER, A. M. J. Mt. Sinai Hosp. **7:** 65, 1940.

PARATHYROID FUNCTION IN THE HYPOPHYSECTOMIZED RAT

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The production of gross and cellular enlargement of the parathyroid glands of a variety of animals by anterior hypophyseal extracts has led to the assumption that the hypophysis exercises a parathyrotropic function (1). Insufficient evidence has been adduced that these extracts produce any hyperparathyroidism. Some degree of rise in blood calcium level has been reported following their administration (1, 2) but this effect has not been observed by all investigators (3, 4). Furthermore, a fall in serum inorganic phosphate, a characteristic parathyroid hormone effect (5), has not been observed (2, 4, 6).

Although parathyroid atrophy has been found in hypophysectomized animals (1), evidence has not been produced that these glands are functionally insufficient. The serum calcium of hypophysectomized mammals is not significantly depressed (1, 6) nor is the serum inorganic phosphate raised (7). The fall in serum calcium of the hypophysectomized amphibian, Xenopus laevis, has not been shown to be mediated through the parathyroids. Ovariectomy also causes hypocalcemia in this species (8).

Some degree of functional impairment of the hypophysectomized animal's parathyroids might conceivably be present but not be manifest under ordinary experimental conditions. Partial parathyroidectomy may produce slight or no change in the serum calcium and phosphate of animals on a normal diet. The present experiment was designed to test the parathyroid function of hypophysectomized rats subjected to the stress of a low calcium diet. At the same time the susceptibility of hypophysectomized rats to parathyroid insufficiency was determined on account of the possibility that hypophyseal insufficiency might modify in some manner the usual response to parathyroid deprivation (cf. the effect of hypophysectomy on the diabetes of pancreatic ablation).

EXPERIMENTAL METHODS. Male albino rats of ages 60–70 days were used. Hypophysectomy was done by the usual parapharyngeal approach. The completeness of operation was checked at autopsy by making serial sections of any tissue remaining in the pituitary fossa and by body weight curves and the size of the adrenals and testes. Animals failing to satisfy all the criteria were discarded. Parathyroidectomy was done under binocular magnification with removal of as little thyroid as necessary. Occasional animals failing to show the characteristic profound change in blood chemistry were proven to have parathyroid remnants by serial section of the remaining thyroid tissue at autopsy. The double operation (both parathyroidectomy and hypophysectomy) was done on one group of rats at the same time. All animals, both operated and controls, were kept in wire-bottom cages maintained at a temperature of 28–32°C. throughout the duration of the experiment. A recovery period of one to three days after

operation was allowed on the normal diet. The times in the table refer to the period on the experimental diet. The animals were killed at intervals from one to four weeks by anesthetizing with ether and bleeding from the abdominal aorta. The blood was collected directly into centrifuge tubes. Serum calcium was determined on 1.0 ml. serum by the method of Clark and Collip. Serum inorganic phosphate was determined on 0.5 ml. serum by the method of Fiske

TABLE 1

Influence of low calcium diet on the serum calcium and inorganic phosphate of hypophysectomized animals and various controls at weekly intervals

DIET	TIME	NO. RATS	SERUM CALCIUM (MGM.%)			SERUM INORGANIC PHOSPHATE (MGM.%)		
			Lowest	Mean	Highest	Lowest	Mean	Highest
Non-operated controls								
	wks.							
Normal...............	4	5	10.6	*10.9*	11.6	5.5	*7.0*	8.0
Low calcium..........	1	4		*10.5*		7.3	*8.0*	8.6
Low calcium..........	2	5	10.5	*10.8*	11.2	5.3	*7.6*	8.5
Low calcium..........	3	11	9.9	*10.4*	10.8	6.5	*8.0*	9.7
Low calcium..........	4	13	7.7	*9.9*	11.6	6.0	*7.0*	9.4
Low calcium..........	1–4	33		*10.3*			*7.5*	
Parathyroidectomized								
Low calcium..........	1–2	6	5.2	*6.0*	7.7	12.0	*12.9*	14.8
Low calcium..........	3	6	5.0	*6.1*	7.3	12.5	*13.1*	13.5
Low calcium..........	4	6	4.5	*5.4*	6.4	13.5	*16.9*	19.0
Low calcium..........	1–4	18		*5.8*			*14.3*	
Hypophysectomized								
Normal...............	4	4	11.0	*11.2*	11.2	4.7	*5.3*	5.6
Low calcium..........	1	8	9.3	*10.6*	11.0	4.6	*6.8*	8.0
Low calcium..........	2	9	9.9	*10.6*	11.4	4.1	*5.0*	6.1
Low calcium..........	3	9	9.7	*10.3*	11.0	5.0	*5.4*	6.1
Low calcium..........	4	17	9.5	*10.5*	11.6	3.4	*4.7*	6.7
Low calcium..........	1–4	43		*10.5*			*5.3*	
Double-operated								
Low calcium..........	1–2	8	4.9	*6.3*	7.1	14.0	*14.9*	15.4

and Subbarow. Individual analyses were made on each rat in most instances. When insufficient serum was obtained for this, analysis was occasionally done on pooled samples. Only occasionally was it not possible to obtain both calcium and phosphate values on the same animal.

The experimental low calcium diet is one which causes marked parathyroid enlargement in rats of this age (9): Alcohol extracted beef fibrin,[1] 20 per cent;

[1] Washed, ground and dried beef fibrin was generously supplied by Dr. J. D. Porsche, Chemical Research Department, Armour & Co., Chicago. It was extracted by refluxing with 95 per cent ethyl alcohol on the steam bath and filtered while hot to remove any vitamin D.

modified Wesson salts,[2] 1.2; KH_2PO_4, 1.8; carotene in oil, 0.1; Wesson oil, 1.9; rice bran extract,[3] 5.0; cane sugar up to 100.0 per cent. This diet contains approximately 0.05 per cent calcium and 0.45 per cent phosphorus almost all of which is inorganic. The normal control diet has the same composition with the addition of 2.5 per cent $CaCO_3$. It maintains normal parathyroid size in rats of this age (9).

RESULTS. The table shows that the serum calcium of the *intact* rats is only slightly lower after 4 weeks on the low calcium diet than that of the controls and that there is no significant difference in the serum phosphates. In marked contrast to this, the *parathyroidectomized* rat has a serum calcium little more than half the normal and a serum phosphate about twice normal. This alteration has taken place before the end of the first week although it tends to become a little more marked in 4 weeks. In spite of this chemical evidence of severe hypoparathyroidism, these rats seldom had manifest tetany.

The *hypophysectomized* rats on the normal diet had a normal serum calcium and a significantly *lower* serum phosphate than the intact animals. We have previously observed this fall in serum phosphate in starved intact animals. It may be a non-specific effect of inanition. It is opposite to the change characteristic of hypoparathyroidism. The low calcium diet produced a very slight fall in the serum calcium of the hypophysectomized rats and no significant difference in serum phosphate after four weeks. The hypophysectomized rats behaved exactly like the intact rats, therefore, in response to the low calcium diet.

Finally, the effect of simultaneous *hypophysectomy and parathyroidectomy* is shown in the last group in the table. The animals withstood the double operation well for the first few days but mortality in this group was high from 5 to 10 days after operation. It was not found practicable to keep them under these experimental conditions for more than two weeks. Within this period of time they had a marked drop in serum calcium and an equally marked rise in serum phosphate. Their reaction was thus indistinguishable from that of the group simply parathyroidectomized.

The average serum protein concentration of 5 hypophysectomized rats kept 4 weeks on the low calcium diet was 5.25 per cent as estimated by the falling drop method. This is a small but significant fall compared to an average of 6.44 per cent in 10 normal rats of the same age on a stock diet. The maintenance of a normal serum calcium is therefore not dependent on a rise in serum protein concentration in the hypophysectomized animals.

DISCUSSION. The functional adequacy of the parathyroid glands of the hypophysectomized rat is well attested by their ability to maintain the serum calcium and inorganic phosphate concentrations within normal limits under the stress of a low calcium diet. No difference has been found between rats hypophysectomized for four weeks and non-operated controls in this respect. At the same time the hypophysectomized rat has been shown to be highly susceptible to para-

[2] Modified to omit calcium and phosphorus salts.

[3] Galen "B", containing vitamins B_1, B_2, B_6, nicotinic acid, pantothenic acid, filtrate factor (The Galen Co., Inc., Berkeley, Calif.).

thyroid deprivation. Since it is known that the parathyroids of intact rats on a low calcium diet of this sort are greatly enlarged, it may be inferred that the parathyroids of the hypophysectomized rat possess a considerable functional reserve.

These results cast a serious doubt on the existence of a direct physiological regulation of the parathyroid glands by the hypophysis analogous to the control exercised over the gonads. Other explanations may be invoked to account for most of the phenomena previously interpreted as evidence of such a function. The atrophy of the parathyroid glands in hypophysectomized animals, for example, is part of a generalized atrophy of organs in these animals (10). Baker (11) found no cytological disturbances in the atrophic parathyroids of the hypophysectomized monkey. It has been suggested by Houssay and Sammartino (12) that this atrophy was due to a generalized nutritional disturbance since it occurred also in their pancreatectomized dogs. It may be significant that our hypophysectomized rats have shown a progressive fall in serum phosphate. A reduction of serum phosphate by restriction of dietary phosphorus is accompanied by atrophy of the parathyroids in rats (13).

Conversely, it may be doubted that the parathyroid enlargement following the injection of hypophyseal extracts is necessarily the direct result of a specific hormonal stimulus. The parathyroid size is remarkably sensitive to dietary alterations and probably to other metabolic factors. It may be significant that very substantial increases in serum inorganic phosphate have been reported after the injection of some hypophyseal extracts (6, 14). The parathyroid enlargement consequent upon renal failure with phosphate retention is a familiar phenomenon and the hyperphosphatemia produced by dietary means may be accompanied by parathyroid enlargement even in the absence of hypocalcemia (13). It would be pertinent also to question the effect of an increase in general metabolism on parathyroid size.

The facts up to this point might be explained on the assumption that hypophyseal secretion increases the demand on the parathyroids. It is not so apparent how a rise in serum calcium following the injection of hypophyseal extracts could be explained on this assumption. However, this reaction has not been observed uniformly and the lack of simultaneous serum protein determinations restricts the interpretation of those results. The efforts made to prove that the reaction was mediated through the parathyroids (1) cannot be regarded as conclusive.

SUMMARY

Evidence has been presented that the function of the parathyroid glands is unimpaired in the young mature male rat deprived of its hypophysis. The hypophysectomized animal maintained its serum calcium and inorganic phosphate concentrations within normal limits as well as the non-operated control even when subjected to the stress of a low calcium diet. In contrast, the hypophysectomized-parathyroidectomized animal under the same conditions suffered a profound fall in serum calcium and rise in serum phosphate, quite like the

parathyroidectomized control. The significance of the parathyroid atrophy following hypophysectomy and of the influence of anterior hypophyseal extracts on parathyroid size and serum calcium, reported by other investigators, has been discussed.

REFERENCES

(1) HOUSSAY, B. A. New England Med. J. **214:** 1128, 1936, lit. cit.

(2) FRIEDGOOD, H. B. AND R. McLEAN. This Journal **118:** 588, 1937.

(3) DIXON, T. F. Biochem. J. **27:** 410, 1933.

(4) SNYDER, F. H. AND W. R. TWEEDY. Proc. Soc. Exper. Biol. and Med. **47:** 234, 1941.

(5) ALBRIGHT, F. Glandular physiology and therapy. Chicago, 1942.

(6) GERSCHMAN, R. AND A. D. MARENZI. Rev. Soc. Argent. Biol. **11:** 500, 1935.

(7) GERSCHMAN, R. C. r. Soc. Biol. **108:** 494, 1931.

(8) SHAPIRO, H. A. AND H. ZWARENSTEIN. J. Exper. Biol. **10:** 186, 1933.

(9) CARNES, W. H. AND H. C. STOERK. Data to be published.

(10) SMITH, P. E. J. A. M. A. **88:** 158, 1927.

(11) BAKER, B. L. Anat. Rec. **83:** 47, 1942.

(12) HOUSSAY, B. A. AND R. SAMMARTINO. C. r. Soc. Biol. **114:** 729, 1933.

(13) CARNES, W. H., A. M. PAPPENHEIMER AND H. C. STOERK. Proc. Soc. Exper. Biol. and Med. **51:** 314, 1942.

(14) CANNAVÓ, L. Biochem. Ztschr. **245:** 234, 1932.

THE REVERSIBILITY OF THE INHIBITION OF RAT BRAIN AND KIDNEY METABOLISM BY COLD[1]

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A new interest in the physiological effects of hypothermia on homeothermic animals has developed as a consequence of the work of Smith and Fay (cf. 1), which has shown that body temperatures of the order of 24°C. can be maintained in human beings for some time without ill effects. That further reduction in body temperature would be unwise is suggested by various observations. Available data indicate that non-hibernating mammals usually do not survive if their body temperature falls to a level of 15° to 20°C. Critical levels of hypothermia have been reported as about 13 to 16° for the rat (2); 20° for the rabbit (3); 16° for the cat (4); 14° (one case) for the monkey (5) and 22° for the dog (6). While the lethal level of hypothermia is thus fairly clear for the animals mentioned, the cause of death in the non-hibernating mammal on acute exposure to cold is not clear. It has been noted by several investigators that in the animal dying of hypothermia the heart continues to beat for some time after cessation of respiration (4, 5, 6). However, it is possible that the respiratory failure is consequent upon impaired circulation. It has been shown that a critical fall in arterial pressure occurs in the dog at about 22°C. (7). Anoxia so established would lead to failure of medullary and other nervous centers (cf. Gerard, 8). It is also possible that death is due to direct and irreversible effects of cold on the brain rather than to failure of the supply mechanisms such as respiration and circulation. The experiments reported here were designed to throw some light on this latter possibility. Besides the work on excised brain, some measurements of respiration were also made on kidney cortex slices to see how this organ, which appears to have the highest metabolic rate of the several organs of the rat (9), would be affected by profound cooling.

METHODS. Five adult rats of the Slonaker-Wistar strain were used. They were killed by decapitation. Measurements of oxygen consumption and of anaerobic glycolysis were made by manometric methods, which have been described in previous communications (10). Procedures peculiar to the problem in hand were these. Oxygen consumption or anaerobic glycolysis was measured for an hour in a thermostat at 37.7° ± 0.01°C. The respirometers were then transferred to another thermostat maintained at 0.2° ± 0.02°C. Fifteen minutes were allowed for thermoequilibration, then readings were taken every fifteen minutes, as before, at this new and lower temperature level. In four experiments the respirometers were transferred back to the first thermostat (at 37.7°C.) after one hour at 0.2°C. (75 min. if the thermoequilibration period be

included). Again fifteen minutes were allowed for thermoequilibration and readings were taken for the ensuing hour. In one experiment, using cerebral cortex slices from one animal, respirometers were kept in the cold bath at 0.2°C. for periods of one, three, five, seven and twenty-four hours before being restored to the warm bath. Points to note are that the rates of cooling and rewarming were kept constant and that the duration of exposure to cold was constant with the exception just described.
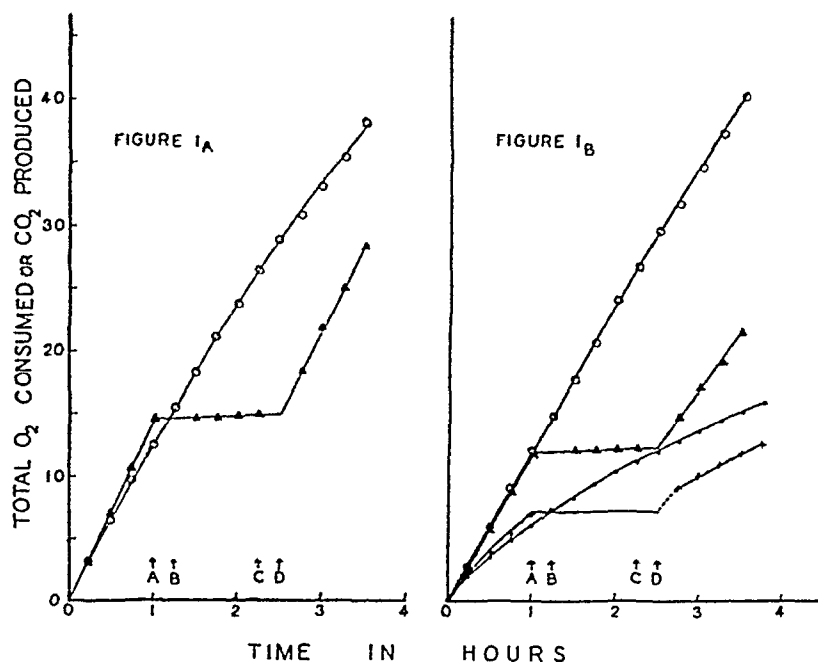
Fig. 1-A. Graph showing the total oxygen consumed, in cubic millimeters, N. P. T., per milligram dry weight by kidney cortex slices as a function of time and temperature. O = control. Total oxygen consumed. Temperature constant at 37.7°C. ▲ = experimental. Total oxygen consumed. Temperature changed as indicated by arrows. A: experimental vessels transferred to 0.2°C. B: end of thermoequilibration period at 0.2°C. C: experimental vessels returned to 37.7°C. D: end of thermoequilibration period at 37.7°C.

Fig. 1-B. Graph showing total oxygen consumed and total carbon dioxide produced (anaerobic) in cubic millimeters, N. P. T., per milligram dry weight, by rat cerebral cortex slices as a function of time and temperature. O = control. Total oxygen consumed. Temperature constant at 37.7°C. ▲ = experimental. Total oxygen consumed. Temperature changed as indicated by arrows. ● = control. Anaerobic glycolysis. × = experimental. Anaerobic glycolysis. A, B, C, D, same as in figure 1-A.

When results are given as rates (differential curves) they are expressed in cu. mm., N.P.T., of oxygen (respiration) or of carbon dioxide (anaerobic glycolysis) consumed or produced per mgm. dry weight per hour (Q_{O_2} and $Q_G^{N_2}$ respectively). When given in terms of total gas consumed or produced as a function of time (integral curves) the units on the Y-axes are cu. mm. gas, N.P.T., per mgm. dry weight.

RESULTS. 1. *Kidney cortex.* It is shown in figure 1-A that the respiration of rat kidney cortex slices drops to a very low level at 0.2°C. This level is fully established at the end of the fifteen minute equilibration period and does not

change, within the limits of error of our measurements, for the ensuing hour. When the tissue is returned to the bath at 37.7°C. there is complete recovery of the capacity for oxygen consumption. In fact, the respiration of the rewarmed tissue is at a somewhat greater rate than that of the control which was not refrigerated. This is perhaps attributable to a decreased catabolism of respiratory catalysts as well as of other substrate at the low temperature.

2. *Brain cortex.* a. *Oxygen consumption.* It is shown in figure 1-B that there is very complete recovery of the respiratory capacity of rat cerebral cortex slices after a period of one hour at 0.2°C. (with 15 min. periods for equilibration). Again, the rate of oxygen consumption is quite constant at the low temperature. However, when the time of exposure to 0.2°C. is lengthened there is progressive diminution in the recovery of oxidative capacity. Thus, after periods of one, three, five, seven and twenty-four hours at 0.2°C., the values of Q_{O_2} obtained during one subsequent hour at 37.7° were 8.50; 7.75; 7.35; 7.22 and 4.43 respectively. The control value (first hour at 37.7°) was 9.07 (each of these values is the mean of determinations run in duplicate). In every case the values of Q_{O_2} were constant at the levels given for the one hour after rewarming during which readings were made.

b. *Anaerobic glycolysis.* It is shown in figure 1-B that recovery of the capacity for anaerobic glycolysis after one hour at 0.2° can be regarded as complete. Insofar as these data are concerned, there is no evidence that anaerobic glycolysis is more liable to damage by cold than respiration. It is interesting that the same "preservation" effect obtained with kidney cortex respiration is obtained here. Thus the rate of anaerobic glycolysis after rewarming, which is during the third hour of the run, is about that of the control during the second hour at 37.7° and definitely greater than the rate of the control during the third hour at 37.7°.

DISCUSSION. As far as we know, neither the respiration and anaerobic glycolysis of mammalian cerebral cortex slices nor the respiration of mammalian kidney cortex slices have previously been measured during and after exposure to a temperature as low as 0.2°C. There are data on the respiration of minced rat brain over the temperature range 25° to 45°C. (11) and on rat cerebral cortex slices over the range 5° to 42°C. (12). The only other mammalian cell species for which we have found similar observations in the literature is sperm. Chang and Walton (13) have shown that if the temperature of a suspension of ram sperm be lowered gradually (5°C. stages with an interval of two hours at each stage), the cells can be cooled to 1°C. and kept at this level for twenty-four hours without loss in respiratory capacity. Using other criteria of recovery than regained respiratory capacity it has been shown that a certain percentage of mammalian sperm can survive exposure to much lower temperatures (14, 15). In view of the findings of Chang and Walton (13), it is possible that the reduction of Q_{O_2} observed in our twenty-four hour run would not have occurred if the cooling had been more gradual.

However, it is shown by our experiments that rat cerebral cortex slices can recover their initial capacity for oxygen consumption and for anaerobic glycolysis after an hour of exposure to a temperature of 0.2°C. even when the cooling and

rewarming are so rapid that they are complete in fifteen minutes or less. This temperature is far below the lethal level for the intact rat, which is about 13° to 16°C. (2). If respiration of the brain as a function of temperature follows the same course *in vivo* and *in vitro*, and if recovery of a normal level of oxygen consumption implies recovery of other functions of the brain, then it follows that death of the intact rat in acute severe hypothermia is not attributable to direct and irreversible action of cold on the brain but rather to failure of the supply mechanisms, circulation and respiration. Irreversible damage in the intact animal is probably due to anoxia of the central nervous system resulting from this failure. In contrast to this picture of failing oxygen and nutrient supply to the brain *in vivo* at low body temperatures stands the continued adequacy of the supply of oxygen and glucose to the tissue slices in the respirometer vessels. Thus it is that the intact rat dies at 13° to 16°C., while the cerebral cortex slice in the respirometer does not show impaired oxidative capacity even after an hour at 0.2°C.

SUMMARY

1. The rate of oxygen consumption of rat kidney cortex slices, measured for an hour at 37.7°C., then for an hour at 0.2°C., was restored to the initial level on rewarming to 37.7°. The periods of cooling and of rewarming did not exceed fifteen minutes each.

2. The rates of oxygen consumption and of anaerobic glycolysis of rat cerebral cortex slices, determined under the conditions described above, also showed full recovery on rewarming.

3. When the duration of the period of exposure of brain slices to 0.2°C. was increased to three, five, seven and twenty-four hours, there was progressive decrease in the rate of oxygen consumption regained on rewarming.

4. The significance of these observations in relation to the death of non-hibernating mammals from acute hypothermia was discussed.

REFERENCES

(1) TALBOTT, J. H. New England J. Med. **224**: 281, 1941.
(2) HAMILTON, J. B. Yale J. Biol. Med. **9**: 327, 1937.
(3) WALTHER, A. Virchow's Arch. **25**: 414, 1862.
(4) BRITTON, S. W. Quart. J. Exper. Physiol. **13**: 55, 1922.
(5) SIMPSON, S. J. Physiol. **28**: Proc. xxxvii, 1902.
(6) WOODRUFF, L. M. Anesthesiology **2**: 410, 1941.
(7) HOOK, W. E. AND R. T. STORMONT. This Journal **133**: P. 146, 1941.
(8) GERARD, R. W. Arch. Neurol. and Psychiat. **40**: 985, 1938.
(9) FIELD, J., 2D, H. S. BELDING AND A. W. MARTIN. J. Cell Comp. Physiol. **14**: 143, 1939.
(10) FUHRMAN, F. A. AND J. FIELD, 2D. J. Pharmacol. and Exper. Therap. **77**: 229, 1943.
(11) HIMWICH, H. E., K. M. BOWMAN, J. F. FAZEKAS AND W. GOLDFARB. Am. J. Med. Sci. **200**: 347, 1940.
(12) FUHRMAN, F. A., N. HOLLINGER, J. M. CRISMON, J. FIELD, 2D AND F. W. WEYMOUTH. Physiol. Zool., in press.
(13) CHANG, M.-C. AND A. WALTON. Proc. Roy. Soc. B**129**: 517, 1940.
(14) LUYET, B. J. AND P. M. GEHENIO. Life and death at low temperatures. Normandy, Missouri, Biodynamica, 1940.
(15) HOAGLAND, H. AND G. PINCUS. J. Gen. Physiol. **25**: 337, 1942.

THE EXCRETION OF SULFANILAMIDE AND ACETYLSULFANILA-MIDE BY THE KIDNEY OF THE RABBIT[1,2]

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Sulfanilamide is eliminated from the mammalian body almost entirely through the urinary excretory system. It appears in the urine of most mammals in two forms. One is the "free" or uncombined form and the other is the "conjugated" or combined form. Combined sulfanilamide has been shown to be acetylsulfanilamide (1). Stewart and collaborators (2) have shown that in the rabbit this acetylation takes place in the liver.

At the present time there are few data available concerning the mechanism by which the kidney excretes sulfanilamide. Marshall and co-workers (3) have studied the mode of excretion of this compound in dogs by comparing its renal clearance with the simultaneous clearance of creatinine. Their studies suggest that sulfanilamide is filtered through the glomerulus and partially reabsorbed, through the tubule. During the course of a study on the excretion of urea and urea derivatives, Nichols and Herrin (4) obtained sulfanilamide clearances which by similar interpretation would lead to essentially the same conclusions as those of Marshall and co-workers. Stewart and associates (5) using a modified clearance technique on humans, and Marshall and co-workers (3) using dogs, found that acetylsulfanilamide has a considerably higher clearance than does the free drug.

The present study is concerned with the mechanism by which the kidney of the rabbit excretes sulfanilamide and acetylsulfanilamide and the factors which influence the excretion of these compounds. The method used involves comparing the renal clearance of these drugs with the simultaneous clearance of one or more compounds the mechanism of excretion of which has been established.

METHODS. Five full grown male rabbits were used in this study. The animals were fasted for at least eight hours previous to each experiment, but they were allowed to drink water as desired.

The unanesthetised animal was tied on his back on the animal board and a number 18 or 20 plain rubber catheter was inserted in the bladder. It remained in the bladder throughout the experiment. The drugs used were either sulfanilamide or acetylsulfanilamide plus inulin, creatinine and diodrast.[3] They were dissolved in 0.9 per cent saline so that the concentration of the compounds was about 400 mgm./100 cc. each of sulfanilamide or acetylsulfanilamide, creatinine

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[1] This study was made in partial fulfilment of the requirements for the degree of Doctor of Philosophy by T. A. Loomis.

[2] This investigation was aided by a grant from the Junior Board of the Buffalo General Hospital.

[3] The diodrast used in this work was kindly furnished by the Winthrop Chemical Company.

and inulin, and about 75 mgm./100 cc. of diodrast. This solution was administered to the rabbit by gravity through a hypodermic needle inserted in a marginal ear vein. The rate of administration of the fluid was controlled by a screw clamp on the rubber tubing below a glass drip indicator. In order to obtain rapidly a desired concentration of the drugs in the blood, the infusion was allowed to run into the vein at a rate of about 5 cc. per minute for ten minutes. Following this a rate of approximately 1 cc. per minute was maintained throughout the remainder of the experiment.

At the end of the rapid infusion period, the bladder was washed with 20 cc. of warm normal saline by means of a syringe attached to the end of the catheter. In exactly 30 minutes the bladder was washed again and the washings were added to the urine formed during the 30-minute period. This was repeated at the end of a second 30-minute period so that two clearance periods were obtained in each experiment. A blood sample was taken at the midpoint of each period by intracardiac puncture and was immediately centrifuged. Potassium oxalate was used in a few of the experiments, but heparin was the anticoagulant usually employed.

All chemical analyses were carried out on a common protein-free plasma filtrate. The filtrate was prepared according to the second zinc sulfate-sodium hydroxide method of Somogyi (6). The drug levels were determined in the plasma filtrates and the urine samples by the same methods. Sulfanilamide and acetylsulfanilamide were determined by the method of Bratton and Marshall (7), inulin by the method described by Hubbard and Loomis (8), creatinine according to Langley's method (9), and diodrast by Alpert's method (10). It was found that recoveries of added inulin, creatinine, sulfanilamide and acetylsulfanilamide from plasma were identical whether the filtrate was made with Somogyi's agents or with the precipitating agents originally suggested by the authors of the various methods. It was also shown by appropriate experiments that the presence of all of these drugs in a common plasma filtrate in the concentration used in the experiments did not interfere with the determination of any one of them.

The clearance of the drugs was calculated according to the formula UV/P, where U and P equal the respective urine and plasma concentrations of the drug, and V equals the urine flow in cubic centimeters per minute.

RESULTS. The results of 14 periods in which the clearances of sulfanilamide, creatinine, inulin and diodrast were determined simultaneously are summarized in table 1. In ten of these periods sulfanilamide was injected and in four periods acetylsulfanilamide was injected and the determinations were based on measurable amounts of free sulfanilamide formed in the animal. Table 1 also summarizes the results of 28 simultaneous clearance periods of acetylsulfanilamide, creatinine, inulin and diodrast. Nineteen of these periods were obtained after administering preformed acetylsulfanilamide and 9 after it was formed by the animal from sulfanilamide. The mean clearances of acetylsulfanilamide and creatinine are almost identical (4.9 and 4.8 cc. per min. respectively). Although the mean clearance of inulin (3.9 cc. per min.) is lower than that of

either of the two former compounds, statistical analysis of the series reveals that the mean clearance of inulin is not significantly different from the mean clearance of creatinine or acetylsulfanilamide.

The clearances of both sulfanilamide and acetylsulfanilamide are independent of the urine flow which varied in the experiments from 0.2 to 1.2 cc. per minute. The clearances of these two compounds are also independent of their plasma concentrations, and it was shown that the rate of excretion (urine concentration times urine flow) of both of these drugs increased when their plasma concentration is increased. During the course of the study the plasma concentration range of sulfanilamide was 1 to 25 mgm. per cent and of acetylsulfanilamide was 0.1 to 6.0 mgm. per cent.

DISCUSSION. Marshall and associates (3) have shown that sulfanilamide is filterable through membranes which are impermeable to the plasma proteins and this has been confirmed in this laboratory. However, there is some evidence that about 20 per cent of the sulfanilamide present in normal plasma is bound to the plasma proteins (11, 12). This would leave only 80 per cent of the drug free to pass through the glomerular membrane. The observed differences between the clearances of inulin or creatinine and sulfanilamide (the ratio of the inulin or creatinine clearance to the sulfanilamide clearance is approximately 3 to 1) is much too great to be explained by the presence of a non-filterable fraction of the magnitude reported. Since the renal clearance of inulin is generally accepted as a measure of the glomerular filtration rate in all mammals in which this has been studied, and since Kaplan and Smith (13) found that the creatinine and inulin clearances are identical in the rabbit, the observed ratio of the inulin or creatinine to the sulfanilamide clearances suggests that sulfanilamide passes through the glomerular membrane and is partially reabsorbed through the tubule from the glomerular filtrate.

During the course of this study it was found that the rabbits were not only able to acetylate sulfanilamide but they were also capable of hydrolysing acetylsulfanilamide after its administration. They were not all able to hydrolyse it to the same extent, consequently some plasma analyses showed only traces of the free drug. Four clearance periods were obtained when it was formed following administration of the conjugated drug, and the mean clearance of sulfanilamide in these four periods (2.3 cc. per min.) was about the same as the mean clearance obtained (1.8 cc. per min.) when the free drug was given directly.

In order to interpret the data which was obtained on acetylsulfanilamide it was necessary to devise an experiment to determine whether this compound will pass through the glomerular membrane. The following experiment was done: acetylsulfanilamide was added to fresh rabbit plasma and the solution was filtered under reduced pressure through a sheet of number 600 cellophane. Repeated determinations showed that the filtrate was protein-free and contained acetylsulfanilamide in the same concentration as the plasma. These results which were similar to the results of Marshall et al. (3) upon sulfanilamide suggest that acetylsulfanilamide in blood plasma can be filtered through the glomerular membrane. The ratio of the mean clearance of acetylsulfanilamide to that of

creatinine and inulin was found to be 1.02 and 1.24, respectively. Therefore, if the creatinine clearance is accepted as a measure of the glomerular filtration rate, the clearance of acetylsulfanilamide is also a measure of that rate. Since evidence indicates that acetylsulfanilamide will pass through the glomerular membrane, these data may be interpreted as meaning that this compound is excreted solely by glomerular filtration. In addition the clearance of acetylsulfanilamide was found to be independent of the plasma concentration of the drug, and its rate of excretion increased with increases in the plasma concentration. These characteristics are true for any compound which is excreted solely by glomerular filtration.

In a manner similar to that shown to be true for sulfanilamide, the mean clearance of acetylsulfanilamide was found to be essentially the same whether it was determined on the preformed compound (19 periods, average clearance is

TABLE 1
Comparison of simultaneous renal clearance periods

PERIODS	COMP. USED	MEAN AND P.E. OF MEAN	MEAN DIFF.* / P.E. OF DIFF.	RATIO OF MEANS
14	S	1.8 ± 0.1	S I = 8.6	S/I = 0.40
	C	6.0 ± 0.3	S C = 11.8	S/C = 0.30
	D	26.1 ± 1.5	C I = 3.0	C/I = 1.31
	I	4.6 ± 0.3		
28	AS	4.9 ± 0.3	AS C = 0.3	AS/C = 1.02
	C	4.8 ± 0.2	AS I = 2.6	AS/I = 1.24
	D	25.2 ± 1.3	C I = 2.4	C/I = 1.23
	I	3.9 ± 0.2		

* In this study the following rule was used: If the mean difference between two columns of figures is 4 or more times the probable error of the difference, this difference is not due to chance but the two means are significantly different.

I = inulin; C = creatinine; D = diodrast; S = sulfanilamide; AS = acetylsulfanilamide.

4.6 cc. per min.) or on the compound formed in the animal from sulfanilamide (9 periods, average clearance is 4.9 cc. per min.).

The diodrast clearance may be used as a reasonably accurate measure of the plasma flow to the kidneys if the plasma concentration of the compound is maintained below a level at which its clearance becomes self depressed (14, 15, 16, 17). The plasma levels were below 8.4 mgm./100 cc. in all experiments in this series. The diodrast clearances at the highest levels attained are in the same range as the clearances obtained when the plasma levels of this compound were 1.0 to 2.0 mgm./100 cc. Thus it appears that the diodrast clearances at the highest plasma levels used in this study were not significantly depressed. The mean clearance of diodrast was found to be approximately 25 cc. per minute. The glomerular filtration rate as determined by the creatinine, inulin or acetylsulfanilamide clearance was approximately 4 to 5 cc. per minute. This means that about one-fifth of the volume of blood plasma delivered to the rabbit's kidney passes through the glomerular membrane and forms the glomerular

filtrate, a figure which is comparable with the proportion of plasma filtered in the dog's kidney as reported by Van Slyke and associates (18).

An attempt was made to determine whether the presence in the plasma of any one of the compounds used in these experiments either increased or decreased the clearance of any of the others. During the course of this study eight clearance periods were obtained in which either two or three of the usual four compounds were given to the animal. In these periods it was found that the clearances were not significantly different from those obtained when all four of the compounds were administered. Thus in these experiments it does not appear that any one of the compounds, in the concentrations which were used, materially influences the clearances of the others.

SUMMARY AND CONCLUSION

1. In rabbits the clearance of sulfanilamide was found to be 30 to 40 per cent of the simultaneously determined clearance of creatinine and inulin respectively. This together with evidence based upon ultrafiltration experiments which indicate that sulfanilamide will pass through the glomerular membrane is interpreted as meaning that sulfanilamide is filtered through the glomerular membrane and partially reabsorbed from the glomerular filtrate.

2. The clearances of acetylsulfanilamide and creatinine were found to be almost identical. Furthermore, the clearance of neither of these compounds differed significantly from the clearance of inulin. This together with evidence based on filtration experiments suggesting that acetylsulfanilamide is free to pass through the glomerular membrane is interpreted as meaning that acetylsulfanilamide is excreted by the rabbit solely by glomerular filtration.

3. The clearances of sulfanilamide and acetylsulfanilamide in this study were found to be independent of their plasma concentration as well as the urine flow.

REFERENCES

(1) MARSHALL, E. K., JR., W. C. CUTTING AND K. EMERSON, JR.   Science **85**: 202, 1937.
(2) STEWART, J. D., G. M. ROURKE AND J. G. ALLEN.   Surgery **5**: 232, 1939.
(3) MARSHALL, E. K., JR., K. EMERSON, JR. AND W. C. CUTTING.   J. Pharmacol. and Exper. Therap. **61**: 191, 1937.
(4) NICHOLES, H. V. AND R. C. HERRIN.   This Journal **135**: 113, 1941.
(5) STEWART, J. D., G. M. ROURKE AND J. G. ALLEN.   J. A. M. A. **110**: 1885, 1938.
(6) SOMOGYI, M.   J. Biol. Chem. **86**: 655, 1930.
(7) BRATTON, A. C. AND E. K. MARSHALL, JR.   J. Biol. Chem. **128**: 537, 1939.
(8) HUBBARD, R. S. AND T. A. LOOMIS.   J. Biol. Chem. **145**: 641, 1942.
(9) LANGLEY, W. D. AND M. EVANS.   J. Biol. Chem. **115**: 333, 1936.
(10) ALPERT, L. K.   Bull. Johns Hopkins Hosp. **68**: 522, 1941.
(11) DAVIS, B. D.   Science **95**: 78, 1942.
(12) DAVIS, B. D. AND W. B. WOOD.   Proc. Soc. Exper. Biol. and Med. **51**: 283, 1942.
(13) KAPLAN, B. I. AND H. W. SMITH.   This Journal **113**: 345, 1935.
(14) SMITH, H. W., W. GOLDRING AND H. CHASIS.   J. Clin. Investigation **17**: 263, 1938.
(15) CHASIS, H., H. A. RANGES, W. GOLDRING AND H. W. SMITH.   J. Clin. Investigation **17**: 683, 1938.
(16) WHITE, H. L. AND P. HEINBECKER.   Proc. Soc. Exper. Biol. and Med. **43**: 8, 1940.
(17) WHITE, H. L., T. FINDLEY, JR. AND J. C. EDWARDS.   Proc. Soc. Exper. Biol. and Med. **43**: 11, 1940.
(18) VAN SLYKE, D. D., A. HILLYER AND B. F. MILLER.   This Journal **113**: 611, 1935.

THE INJURY CURRENT IN THE ELECTROCARDIOGRAM[1]

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Injury to the surface of the mammalian heart produces deviation of the S-T
segment of the electrocardiogram, the direction of which depends upon the
location of the injury. Injury to the right ventricle causes a depression, and
injury to the left ventricle an elevation of the S-T segment with reference to the
diastolic base-line. The extent of the injury determines the degree of elevation
or depression (1).

Consideration of the probable explanation of this phenomenon led to a series
of experiments based on the following assumptions.

1. Injury to the surface of a ventricle leads to the production of an injury
potential.

2. The direction in which the injury potential is oriented in relation to the
leads, and consequently its effect upon the leads, depends upon the orientation in
space of the injured region.

3. Since the injured surface is in the negative field of the injury potential and
the rest of the heart in its positive field, the anatomical relationships would neces-
sitate that the current of injury would cause a downward deflection of the string
when the injury is on the left ventricle and an upward deflection of the string
when the injury is on the right ventricle.

4. During systole the source from which the injury current arises becomes de-
polarized, the injury current disappears and the string should return to the iso-
electric level. Whether the source of the injury current is visualized as the result
of a relative negativity of injured as compared to normal tissue or as the result of
dipoles standing across the "membrane" of demarkation between injured and
uninjured tissue, the electrical changes as they affect remote leads would be the
same.

The habit of considering the diastolic position of the string as the normal base
line or the isopotential level has prevented investigation of this problem, as has
also the difficulty of producing an injury to the heart *in situ* while the electro-
cardiogram is being recorded. The following experiments were designed to
circumvent this difficulty.

METHOD. A string galvanometer was employed for these experiments, be-
cause it will maintain a constant deflection in the presence of a constant po-
tential. Instruments with condenser-coupled amplifiers obscure the phenom-
enon because of the tendency of the beam to return to a center position despite

the presence of constant potentials. Thirteen dogs were employed, prepared as previously described (1).

Experiments of two types were performed. In the first, a small pledget of cotton wool wrung out in Ringer's solution was placed at the apex of the left ventricle or over the conus of the right ventricle. A short segment of fine-bore rubber tube was led into the pledget, and the chest wound closed around it. The animal was placed so that the pledget was on the most dependent portion of the heart. Recording was begun of an electrocardiogram from lead II or III, and continued while 0.5 to 2.0 cc. of M/5 KCl was injected rapidly into the pledget. The regions studied are best represented in those leads.

In the second type of experiment a small pledget (1–2 cm. in diameter), moistened in M/5 KCl, was placed on the apex of the left ventricle or on the conus of the right ventricle. Over it were placed in succession a silver-silver chloride disk electrode (1.0 cm. in diameter), a somewhat larger sheet of dry blotting paper or rubber dam, and finally a second silver-silver chloride electrode. The electrodes were connected by fine insulated wires to a switch, by means of which the insulation between the two plates could, in effect, be removed or applied at will. This was then done while the electrocardiogram was being recorded from lead II or III. The same type of experiment was performed on four animals in which an irreversible injury was produced by a burn at the left apex, and on five in which an infarct was produced by multiple ligation of vessels leading to the apex of the left ventricle.

RESULTS. Figure 1 shows the results of application of M/5 KCl to the surface of the left ventricle (A) and of the right ventricle (B). It can be seen that the actual deviation is that of the base-line, and that the S-T take-off remains at the original iso-electric line. Such fluctuations as are seen in the height of the S-T take-off are interpreted as resulting from respiratory and other changes in the position of the heart. Injury to the right ventricle causes an upward deviation of the base-line, while injury to the left ventricle is followed by downward deviation of the base-line.

Figure 1, C and D, illustrates the results of the second series of experiments, in which insulation over an injured region was temporarily nullified by establishing the circuit between the plates inside and outside of the insulation. Short-circuiting the insulation over a KCl injury of the right ventricle produced an upward deviation of the base-line, and a "depressed" S-T take-off which actually remained at the level of the former base-line (fig. 1C). Restitution of insulation was followed by return of the base-line to its former level and restoration of the normal S-T take-off.

Nullification of insulation over a KCl injury of the left ventricle produced a downward deviation of the diastolic base-line, and an "elevated" S-T take-off which actually remained at the iso-electric level (fig. 1D).

Experiments with burned areas were performed only on the left ventricle. The results were the same as those obtained with KCl on the left ventricle (fig. 2D). Circumvention of the insulation produced a downward deviation of the base-line and an "elevated" S-T take-off, which in fact remained at the iso-electric level. Restoration of the insulation was followed by return of the de-

pressed base-line to its original level and the disappearance of the deviation in the S-T take-off.
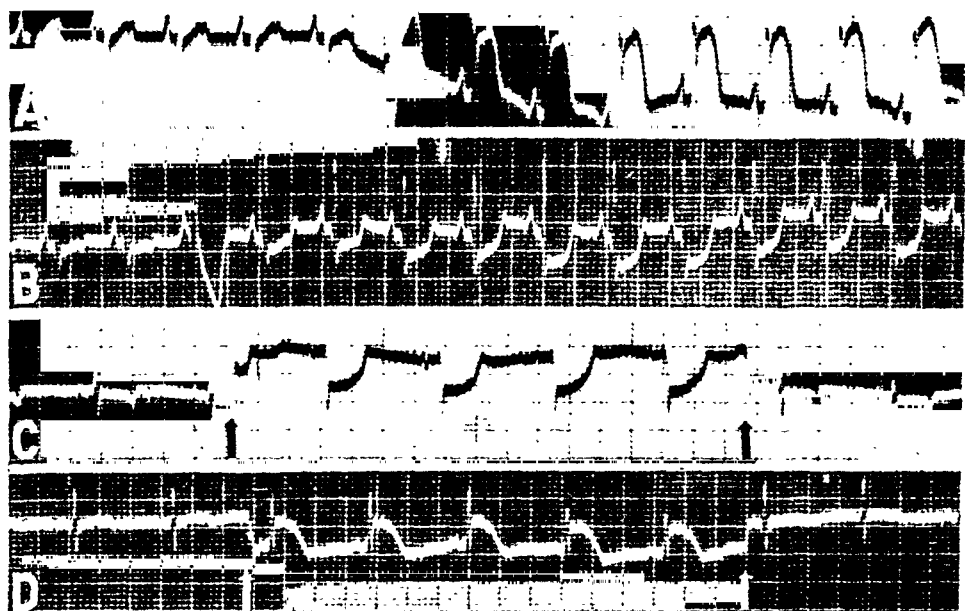
Fig. 1. A, B. July 31, 1942. Dog, 7.5 kgm. Dial anesthesia. A. Animal on left side; 1.0 cc. M/5 KCl injected on to left ventricle. Lead III. B. Animal on right side; 2.0 cc. M/5 KCl injected on to right ventricle. Lead III. C, D. August 6, 1942. Dog, 7.0 kgm. Dial anesthesia. C. Pledget soaked in M/5 KCl on anterior surface of right ventricle. Insulation was short-circuited between arrows. Lead III. D. Pledget soaked in M/5 KCl on left ventricle near apex. Insulation was short-circuited between arrows. Lead III.
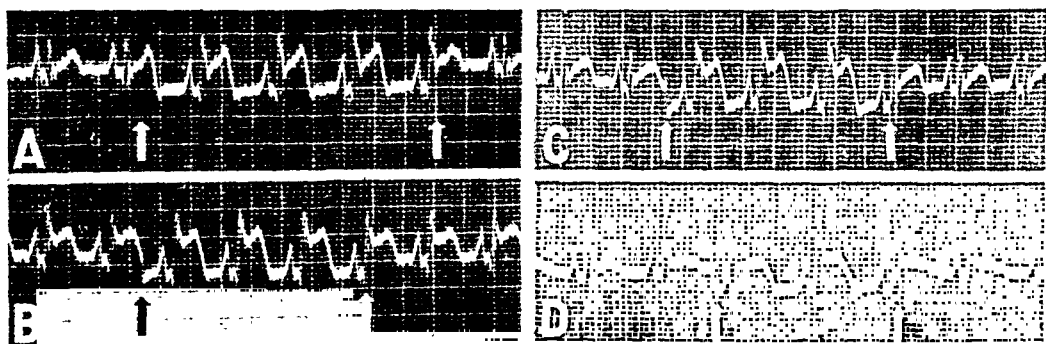
Fig. 2. A, B, C. November 3, 1942. Dog 9.0 kgm. Dial anesthesia. Infarct on posterior surface of left ventricle near apex. A. Small infarct totally insulated by small insulation size of plates (2.0 cm. in dia.). Between arrows the insulation was short-circuited. B. Larger infarct not totally covered by insulation the same size as short-circuiting plates. Between arrows the insulation was short-circuited. C. Large infarct totally covered by insulation much greater in area than the short-circuiting plates. Between arrows the insulation was short-circuited. D. September 9, 1942. Dog, 8.0 kgm. Dial anesthesia. Insulation of burned area on left apex 1.5 cm. in diameter. Between arrows the insulation was short-circuited. All records are lead III.

Figure 2 A illustrates the result of short-circuiting and restoring insulation over an area of infarction at the left apex produced by ligation of coronary arteries. It can be seen that an injury potential is present in myocardial infarction and is

identical with that produced by injury with KCl or burning. The S-T take-off is "elevated" only because the diastolic base-line is depressed. Actually, the S-T take-off remains at the original isopotential level.

It was noteworthy that, whenever the insulation was short-circuited, or restored, during the S-T interval, no displacement of the string occurred. If the insulation was short-circuited at this time, the base-line was displaced only at the end of T. We have records of 10 experiments, of which figure 2A is an illustration. Injury was produced by infarction (4 cases), burning (3 cases) and potassium (3 cases). In no instance was there a change in the tracing coincident with the shunting in or out of the insulation during the S-T period. No current is being generated at the site of injury and whether the insulation shields this region or not can make no difference. Figure 1C is not an exception for the reason that recovery had already begun.

Whether a positive phase of the action potential exists, whether injury is necessary to show it and what the conditions are that make it most manifest, are questions on which the present experiments throw no certain light. Ideas on the subject are confused. Some work indicates that an injured region of the ventricle becomes quite as positive during activity as it is negative during diastole (2). Other experiments performed in a similar fashion indicate that the injured muscle is very much more negative at rest than it is positive during activity (3, fig. 1). Still other work has it that the region of injury (partial potassium depolarization) is negative during rest and iso-electric during activity (4, p. 43).

If the injured region comes into the positive field of action potential during systole and is in the negative field during diastole, it is difficult to see why current in the ordinary leads would not reverse completely from systole to diastole instead of being essentially monophasic. Except for the superimposed QRS deflections the string seems to vary from the iso-electric level in one direction only in any single experiment.

There are apparent exceptions to this rule, as indicated by the following. A large quantity of KCl was infused on to a cotton pledget, laid at the apex of the left ventricle, while the electrocardiogram was being recorded (10–20 cc. instead of 0.5–1.0 cc.). The S-T take-off was then often above the original isopotential line instead of at the same level. Possible explanations are: 1, that injury was sufficient to produce a positive injury action potential; 2, that the potassium solution, spilling generously over the intrathoracic organs, induced injury currents that were not quenched by the systolic depolarization of the heart muscle; 3, that partial injury was produced in other regions of the heart beyond those more completely injured in which depolarization was incomplete but which failed to respond to excitation.

A second set of circumstances gave similar appearances (fig. 2C). A large area of the left ventricle was injured by infarction or by application of KCl. The injured area was shielded by a large insulating layer, so that the expected S-T "elevation" was slight or absent. The plates used to short-circuit the insulation were smaller than insulation or the injured area. When these plates were connected, the S-T take-off was above what it had been before. This may be taken as evidence for a positive injury action potential or may more simply be due to

the fact that when the plates were connected the injury current spread to the leads from a different and more concentrated source than would have been the case if the plate electrode had been co-extensive with the injury.

When a large injured area is partly covered by a sheet of insulating material of relatively small area, the injury current is not wholly shielded from the leads and the S-T segment is "elevated." If two plates just covering the two sides of the insulation are connected and the insulation thus eliminated, the S-T segment remains at exactly the same level and has the same contour as it had before. The injury current can flow more freely now during diastole and the diastolic parts of the record are lowered (see fig. 2B). The fact that variations in the resistance to current flow make no difference in the systolic parts of the curve is compatible with the view that no current flows from the injured region to the leads during systole.

DISCUSSION. These experiments establish the fact that injury to the surface of the heart produces a persistent displacement of the diastolic base-line of the electrocardiogram. This displacement is upward when the right ventricle is injured and downward when the left ventricle is injured. Results of previous experiments in which potentials of known polarity were impressed across the heart between the right and left ventricles have demonstrated that, when an electrode at the surface of the right ventricle is made negative with respect to an electrode at the surface of the left ventricle, there is an upward displacement of the beam, while negativity at the left ventricular electrode causes a downward displacement of the beam (5). It can therefore be inferred that the persistent diastolic deviation of the string in the present experiments is due to the establishment of a persistent potential so oriented that the injured regions are in the negative field and the uninjured parts are in the positive field. Whether this injury current flows between closely placed dipoles at the boundary of injured and non-injured tissue or between the surfaces of the right and left ventricles, the deviation of the string recorded in the lead studied would be expected to be the same.

During systole the injury current disappears, as is shown by the return of the string to the original base-line, and by the absence of displacement of the string when insulation is short-circuited during the S-T interval. At the end of systole the injury current appears. This disappearance and reappearance of the current of injury describes a monophasic curve upon which are superimposed the QRST deflections which result from the activity of normal cardiac muscle.

The S-T take-off therefore occurs at the iso-electric level, indicating at this point the abolition or equalization of potential differences in the portions of the heart represented in the lead studied.

"Elevation" of the S-T segment means in reality, therefore, the depression of the diastolic base-line by a current of injury resulting from injury to the left ventricle.

"Depression" of the S-T segment denotes the elevation of the diastolic base-line by a current of injury resulting from injury to the right ventricle. The degree of displacement is an index of the degree or extent of injury.

Whether the agent producing the injury be KCl, a burn or a myocardial in-

farct, the nature and direction of the injury current are the same. Whether the injury is extremely superficial, as with KCl, or whether it is deep, as with thermocoagulation or an infarct, is likewise without influence on the resulting deviation.

Other factors than the presence of an injury current may give rise to a deviation of the S-T take-off. Examples may be found in electrocardiograms of bundle-branch block, tachycardia, digitalis intoxication and ventricular ectopic beats. In these circumstances terminology may be more clear if we speak of this situation as a superimposition or fusion of QRS and T, rather than deviation of the S-T segment, preserving the latter term for the above described function of the injury current. Differentiation into these two categories should not be difficult except where both phenomena occur together.

These observations permit a more complete statement of a standpoint which has been taken in earlier papers of this series. It was hypothesized that potassium suppressed electrical activity on the surfaces where the material was spread, and that the resulting monophasic deflection was due to the activity of the uninjured portion of the heart. It is now evident that the potassium or other injury suppresses the phasic activity of the injured tissue and produces an injury current. The monophasic deflection is due to suppression of this current, by the activity of tissue which remains capable of phasic response. It is possible that the injury current is quenched only as the whole heart becomes depolarized, but there is no evidence in the present experiments contrary to the idea that the injury current is produced by dipoles standing across the demarkation "membrane" which separates injured from uninjured tissue and that the process of excitation depolarizes this "membrane" locally.

CONCLUSIONS

1. Injury to the right ventricle causes an upward displacement of the diastolic base-line in the three standard leads of the electrocardiogram. Injury to the left ventricle produces a downward displacement of the diastolic base-line.

2. During systole there is a return of the string to the isopotential level which constitutes the S-T segment.

3. In reality, therefore, elevated S-T segments must be interpreted as due to an injury potential resulting from left ventricular damage, which produces in the electrocardiogram a downward displacement of the diastolic base-line.

4. Depressed S-T segments must be interpreted as due to an injury potential resulting from right ventricular damage, which produces in the electrocardiogram an upward displacement of the diastolic base-line.

REFERENCES

(1) HOFF, H. E., L. H. NAHUM AND B. KISCH. This Journal 131: 687, 1941.
(2) EYSTER, J. A. E., W. J. MEEK, H. GOLDBERG AND E. W. E. GILSON. This Journal 124: 717, 1938.
(3) SUGARMAN, H., L. N. KATZ, A. SANDERS AND K. JOCHIM. This Journal 130: 130, 1940.
(4) ASHMAN, R. AND E. HULL. Essentials of electrocardiography. 2nd Edition, New York, Macmillan, 1941.
(5) HOFF, H. E., L. H. NAHUM AND W. KAUFMAN. This Journal 138: 644, 1943.

THE EFFECT OF POSITIVE AND NEGATIVE INTRA-THORACIC PRESSURE ON PERIPHERAL VENOUS PRESSURE IN MAN[1]

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In an earlier paper (Holt, 1941) it was shown that in the anesthetized dog in the supine position peripheral venous pressure is not a function of right auricular pressure when right auricular pressure is decreased. Evidence was presented that this is due to the fact that when right auricular pressure is decreased the veins become partially collapsed just before entering the chest, increase the resistance to the flow of blood along the veins, and maintain peripheral venous pressure constant when right auricular pressure is decreased.

Recently Ryder, Molle and Ferris (1941) have stated that in most normal human subjects peripheral venous pressure is a function of tissue pressure and is independent of right auricular pressure. The experiments reported in this paper were performed in order to determine if peripheral venous pressure is a function of right auricular pressure in man.

METHODS AND RESULTS. Venous pressure was measured in the antecubital vein by a modification of the direct method of Moritz and Tabora (1910) using 0.85 per cent sodium chloride solution in the manometer system. In the supine position the venous pressure was referred to the level of the middle of the chest as zero (von Recklinghausen, 1906). In the sitting position the venous pressure was referred to the lower end of the sternum as zero. As eleven or more venous pressure determinations were made in each experiment, a slow continuous injection of saline was made between each venous pressure determination in order to prevent clotting.

The subject breathed through a mouthpiece from a breathing chamber in which the pressure could be varied from twenty centimeters of water above to twenty centimeters below atmospheric pressure. The breathing chamber had a volume of approximately twenty-five liters. A continuous flow of fifty liters or more of air passed through the chamber each minute in order to keep the air in the chamber about the same composition as that of room air. The pressure in the chamber varied around one centimeter of water with each respiration. The subject breathed from the chamber continuously, and whenever the pressure in the chamber was changed, one to three minutes was allowed before the venous pressure reading was made.

Peripheral venous pressure was measured in eight subjects in the supine position, lying on a flat table, with the arm abducted to approximately forty-five degrees and held well below heart level. The subjects breathed from the cham-

[1] A preliminary report of this work was given at the meeting of the American Federation for Clinical Research in Minneapolis, 1942.

ber in which the pressure was varied from fourteen centimeters of water above atmospheric to fourteen centimeters below. Figure 1 shows the results of a typical experiment. As the chamber pressure was increased, peripheral venous pressure increased and as the chamber pressure was decreased peripheral venous pressure decreased. There was a linear relationship between peripheral venous pressure and chamber pressure as shown in figure 1. It appears that when air under the above negative pressures was breathed there was no collapse of the veins and that peripheral venous pressure was a function of right auricular pressure.

In three subjects the experiment was performed as described above with the exceptions that a maximum inspiratory effort lasting thirty seconds was made against a mercury manometer. In these experiments the intra-thoracic pressure was decreased below atmospheric by forty centimeters of water or more. The peripheral venous pressure fell seven centimeters or less during the first fifteen or twenty seconds and then started to rise. When these large negative intra-
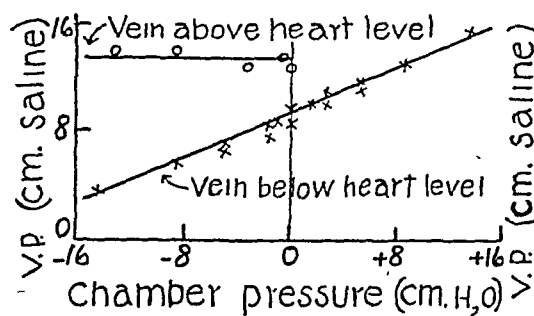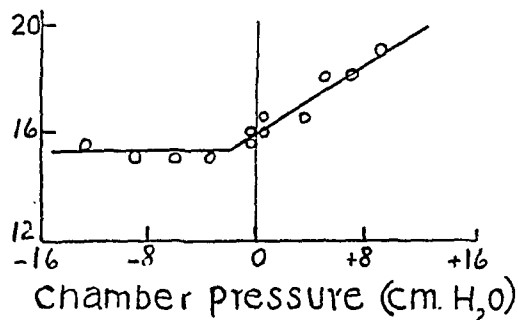
Fig. 1. The effect of changing the breathing chamber pressure on peripheral venous pressure in the supine subject. *V.P.*, venous pressure. +, above atmospheric pressure. —, below atmospheric pressure.

Fig. 2. Same as figure 1 but with the subject in the sitting position.

thoracic pressures were developed, it appears that the peripheral venous pressure fell to a point where the veins became partially collapsed, and beyond this point peripheral venous pressure was independent of right auricular pressure.

In three subjects the experiment was performed as described in the first group of experiments with the exception that the antecubital vein was elevated to a point well above heart level. The peripheral venous pressure, referred to the heart as zero, was increased when the arm was placed in this position. This was due to the fact that the peripheral veins became partially collapsed (Lyons, Kennedy and Burwell, 1938; Holt, 1940). When the subject breathed air that was under a negative pressure the peripheral venous pressure remained constant (fig. 1). Thus when the arm was elevated well above heart level it appears that the peripheral venous pressure was not a function of right auricular pressure because the veins became partially collapsed in the periphery.

It was thought that with the subject in the sitting position the column of blood extending from the upper end of the thoracic cage down to the right auricle

would exert a negative pressure on the veins entering the upper end of the thoracic cage and tend to collapse them.   In order to determine if there was a partial collapse of the veins entering the upper end of the chest, venous pressure was measured in the antecubital vein of three subjects in the sitting position with the arm abducted to approximately forty-five degrees and resting on a board. The subject breathed from the chamber as in the above experiments.   When air under positive pressure was breathed the peripheral venous pressure was increased, and when air under negative pressure was breathed the peripheral venous pressure remained constant (fig. 2).   Thus it appears that when air under a negative pressure was breathed, there was a collapse of the veins between the point where the venous pressure was measured and the right auricle, and that peripheral venous pressure was not a function of right auricular pressure. Since, in the sitting position, the blood ran upward from the point in the vein where the pressure was measured to the upper level of the thoracic cage it would appear that there was not a collapse of the veins in the periphery but that the collapse occurred at the point where the veins enter the upper end of the chest.

DISCUSSION.   Since right auricular pressure was not measured in these experiments, there was the possibility that a change in intra-thoracic pressure was not associated with a change in right auricular pressure.   However, since it has been shown in the dog that right auricular pressure is a linear function of intra-thoracic pressure (Holt, 1941), and since in the supine human with the arm held below heart level a rise in intra-thoracic pressure is associated with a rise in peripheral venous pressure and a decrease in intra-thoracic pressure is associated with a fall in peripheral venous pressure, it would appear that intra-thoracic pressure changes are transmitted to the right auricle.

Although it has been shown in the dog that peripheral venous pressure is not a function of right auricular pressure when right auricular pressure is decreased, the data presented here indicate that in man peripheral venous pressure is a function of right auricular pressure when the subject is supine and the arm is below heart level.   This difference would appear to be due to the fact that in the supine dog right auricular pressure is atmospheric or slightly sub-atmospheric, while in man in the supine position the pressure in the right auricle is several centimeters of saline above atmospheric (Richards et al., 1942).   As a result a small decrease in right auricular pressure in the dog causes the veins to become partially collapsed just before entering the chest, thus making peripheral venous pressure independent of right auricular pressure.   However, in man right auricular pressure may be lowered considerably before the pressure in the veins outside the chest becomes low enough to cause them to become partially collapsed, and until that point is reached peripheral venous pressure is a function of right auricular pressure.

When the arm is elevated several centimeters above heart level in the supine position the veins become partially collapsed peripherally because the pressure inside of them is less than that outside, and as a result changes in right auricular pressure do not cause a change in the peripheral venous pressure.   With the subject in the sitting position the veins appear to become partially collapsed

at the point where they enter the upper end of the thorax and peripheral venous pressure is not a function of right auricular pressure.

There is the possibility in those experiments where peripheral venous pressure remained constant when the intra-thoracic pressure was decreased that as a result of the decreased intra-thoracic pressure cardiac output was increased and the rate of flow of blood along the veins of the arm was increased, thus keeping the peripheral venous pressure constant. This is unlikely because in the supine position with the arm below heart level, the peripheral venous pressure decreased when the intra-thoracic pressure was decreased.

SUMMARY

Venous pressure was determined in the antecubital vein by a modification of the direct method of Moritz and Tabora in eight normal subjects who breathed from a chamber in which the pressure was varied from fourteen centimeters of water above to fourteen centimeters below atmospheric. In the supine subject, with the arm held well below heart level, the peripheral venous pressure decreased when air under negative pressure was breathed, and increased when air under positive pressure was breathed. When the arm was held well above heart level, in the supine subject, the peripheral venous pressure remained constant when intra-thoracic pressure was decreased. In the sitting position the peripheral venous pressure remained constant when the intra-thoracic pressure was decreased.

In normal man, in the supine position with the arm well below heart level and abducted to forty-five degrees, peripheral venous pressure is a function of right auricular pressure.

REFERENCES

HOLT, J. P. This Journal **134**: 292, 1941.
         This Journal **130**: 635, 1940.
LYONS, R. H., J. A. KENNEDY AND C. S. BURWELL. Am. Heart J. **16**: 675, 1938.
MORITZ, F. AND D. VON TABORA. Deutsch. Arch. f. klin. Med. **98**: 475, 1910.
RICHARDS, D. W., JR., A. COURNAND, R. C. DARLING, W. H. GILLESPIE AND E. DeF. BALD-
         WIN. This Journal **136**: 115, 1942.
RYDER, H. W., W. E. MOLLE AND E. B. FERRIS, JR. Proc. Central Soc. Clin. Research **14**:
         44, 1941.
VON RECKLINGHAUSEN, H. Arch. f. exper. Path. u. Pharmakol. **55**: 463, 1906.

ON THE EXCRETION OF IODINE IN THE SALIVA[1]

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The excretion of iodine in the saliva after the oral or parenteral administration of iodine compounds has received comparatively little attention to date. In 1929 Lipschitz (1) showed that in dogs, following the intravenous injection of sodium iodide in doses ranging from 20 to 50 mgm. per kilogram, the parotid saliva attained a concentration of iodine 5 to 7 times that of the blood. Even greater concentrations were observed in the gastric secretion. Barkan and Leistner (2) reported simultaneously that when an organic iodine compound "Jodtropon" was fed to a dog, so-called "organic" iodine failed to appear in either salivary or gastric secretions. In 1930, Lipschitz (3) showed that in dogs only minimal amounts of iodine appeared in the saliva or gastric juice after the intravenous administration of large doses of thyroxine. He assumed that the small amounts of iodine secreted in these body fluids represented inorganic iodine gradually released from the circulating thyroxine.

In 1931, Schneider and Widmann (4) found the concentration of iodine in human saliva to vary from 0 to 362 micrograms per 100 ml. Scheffer (5) also found the excretion of iodine in the saliva to be marked at times. Bruger, Hinton and Lough (6), however, found the iodine content of normal human saliva to vary between 3.5 and 24.0 micrograms per 100 ml. with a mean of 10.2 ± 5.1. Undoubtedly, some of the high figures for human salivary iodine heretofore published were obtained from subjects either ingesting or exposed to abnormal amounts of iodine. In a recent report, Flexner, Bruger and Member (7) demonstrated that in man, after the intravenous injection of sodium iodide, inorganic iodine is selectively concentrated and secreted by the salivary and gastric glands.

Thienes and Hockett (8) studied the rate of intestinal absorption of potassium iodide in man after the subcutaneous injection of an extract of the posterior lobe of the pituitary. The time of appearance of measurable quantities of iodine in the saliva was used to determine the rate of absorption of iodide. Their results indicated that the appearance of iodine in the saliva was delayed 40 minutes after the injection of the pituitary extract. Heath and Fullerton (9), utilizing a spot test to detect iodine in the saliva, found that after the oral administration of 0.25 gram of potassium iodide to 8 normal subjects, iodine appeared in the saliva within 10 to 15 minutes. In 21 patients, all but two showed no detectable salivary iodine after 20 hours. They found the intestinal absorption of iodide to be delayed in a wide variety of clinical conditions including patients who had achlorhydria or who suffered from some disease process likely to condition a disturbance of the absorptive mechanism.

MATERIAL AND METHODS. The present studies were carried out on 16 normal subjects (usual hospital personnel) and on one patient with essential hypertension. Persons assigned to surgery were uniformly excluded because of probable contact with iodine. Unless indicated, the experiments were done in the morning after breakfast. Wide-mouthed bottles used to collect the saliva, after thorough cleaning, were rinsed with iodine-free distilled water. Each subject chewed a small amount of paraffin to insure an adequate flow of saliva; from 1 to 5 ml. were collected at stated intervals.

In the oral experiments, the iodine compound was administered in a gelatin capsule with particular precautions taken to insure that the outside of the capsule was not contaminated with iodine. Approximately 200 ml. of water was

TABLE 1

The iodine content of the saliva (mgm. per 100 ml.) of seven normal subjects following the ingestion of potassium iodide in amounts ranging from 50 to 500 mgm.

(38 to 380 mgm. of iodine)

SPECIMEN	GROUP I 50 MGM. POTASSIUM IODIDE (38 MGM. IODINE)							GROUP II 100 MGM. POTASSIUM IODIDE (76 MGM. IODINE)		GROUP III 500 MGM. POTASSIUM IODIDE (380 MGM. IODINE)	
	D. O.	M. B.	E. McL.	S. M.	M. G.*	M. G.	M. S.	E. McL.	E. McD.	M. G.	M. B.
hours											
0	0.013	0.013	0.015	0.019	0.005	0.014	0.025	0.015	0.008	0.008	0.012
½	1.692	1.226		2.609	2.553						
1	2.547	2.428	2.143	2.861	3.517						
1½	2.646	2.365	1.634	2.366	2.961	2.392	2.168	3.247	9.182	9.770	13.225
2	3.111	1.932	2.345	2.642	3.391						
2½	3.335	2.249	2.446	2.725	2.133						
3		1.737	2.057	2.396	1.846	1.800	1.217	2.462	3.428	7.319	9.845
6		1.912	1.458	2.153	1.400	1.991	0.794	2.120	3.416	8.085	8.055
12		0.741	1.025	1.370	1.000	1.468	0.718	0.990	1.805	6.100	3.037
24		0.177	0.177	0.574	0.155	0.314	0.143	0.388	0.969	1.475	1.489
48		0.028	0.056	0.048	0.045	0.083	0.034	0.063	0.196	0.379	0.314
72		0.011	0.021	0.016		0.018	0.009	0.024	0.040	0.030	0.068
96									0.025	0.008	0.020

* Fourteen hours post-prandial.

imbibed to wash down the capsule. The mouth was then rinsed with a dilute solution of alcohol. Potassium iodide, iodine dissolved in poppy seed oil,[2] ethyl diiodobrassidate[3] and sodium tetraiodophenolphthalein were thus administered. In the parenteral experiments, sodium iodide in aqueous solution was injected intravenously. The method of Trevorrow and Fashena (10, 11) was used throughout.

RESULTS. *Experiments with iodides.* Table 1 shows the salivary iodines of 7 normal subjects before and after the oral administration of 50 to 500 mgm. of potassium iodide (38 to 380 mgm. of iodine). The maximal concentration of iodine in the saliva occurred in 1 to 2½ hours after the ingestion of the iodide;

[2] Lipiodol (Fougera).
[3] Lipoiodine (Ciba).

following 50 mgm. of potassium iodide it reached 700 times that of the control level and after 100 to 500 mgm. 1100 to 1200 times the initial salivary iodine. Abnormal amounts were still detected in the saliva 48 hours following the ingestion of the iodide but after 72 or 96 hours normal values were usually obtained.

In one patient with essential hypertension, whole blood and salivary iodines were done before and at frequent intervals (total period of 48 hrs.) after the ingestion of 50 mgm. of potassium iodide (38 mgm. of iodine). Maximal concentrations of iodine in both body fluids were noted at the 2-hour period (0.126 mgm. per 100 ml. of whole blood and 2.974 mgm. per 100 ml. of saliva) but augmented values persisted in the saliva for a longer time. The salivary/blood iodine ratio in the control period was 6 and reached a maximum of 28, 12 hours after the ingestion of the iodide.

TABLE 2

The iodine content of the saliva (mgm. per 100 ml.) of 8 normal subjects following the intravenous administration of sodium iodide in amounts ranging from 25 to 100 mgm. (22.4 to 89.6 mgm. of iodine)

SPECIMEN	GROUP I 25 MGM. SODIUM IODIDE (22.4 MGM. IODINE)		GROUP II 50 MGM. SODIUM IODIDE (44.8 MGM. IODINE)			GROUP III 100 MGM. SODIUM IODIDE (89.6 MGM. IODINE)		
	L. W.*	E. F.*	L. K.*	E. B.*	E. R.*	S. B.	W. K.	S. E.*
minutes								
0	(0.011)	(0.008)	(0.012)	(0.007)	(0.009)	(0.009)	(0.012)	(0.011)
5	3.80	4.00	8.40	3.15	6.70	14.30	6.70	14.30
10	2.90	2.70	5.67	2.94	3.80	11.10	8.20	.
15	2.90	2.30	7.77	2.73	4.40	10.10	7.80	11.60
30	2.10	2.10	9.03	2.52	4.00	12.20	8.80	12.60
45	1.90	2.10	7.77	2.10	3.60	12.00	7.60	10.30
60	1.70	1.70	9.24	1.89	3.80	8.20	5.70	8.60
90	1.50	1.50	9.24	1.68	2.90	10.10	7.80	8.60
120	1.40	1.30	6.30	1.89	2.50	7.40	4.80	7.00

* Fourteen hours post-prandial.

Table 2 demonstrates the salivary iodines of 8 normal subjects before and after the intravenous administration of 25 to 100 mgm. of sodium iodide (22.4 to 89.6 mgm. of iodine). The appearance of high concentrations of iodine in the saliva as early as 5 minutes after the injection was noted. In fact, the greatest concentration occurred at that time, then gradually tapered off but iodine was still present in the saliva in appreciable amounts two hours after the injection. Apparently, iodine was secreted in greater concentration in the saliva following intravenous injection than after the oral administration of an equivalent amount of iodide.

Experiments with iodine dissolved in poppy seed oil. In six studies on four normal subjects, salivary iodines were determined before and at frequent intervals (total period of 168 hrs.) after the ingestion of a capsule containing 200 mgm. of iodine dissolved in poppy seed oil. The increased values for salivary iodine were not unlike those obtained with equivalent amounts of iodine as

potassium iodide.   Concentrations of iodine in the saliva as high as 5.0 or 6.0 mgm. per 100 ml. were reached occasionally.   In 3 experiments, no food was ingested for 14 hours prior to the investigation but this factor appeared to exert little influence on the concentration of iodine in the saliva.

Experiments with ethyl diiodobrassidate.   In one normal subject, 123 mgm. of iodine as ethyl diiodobrassidate was ingested on three separate occasions (adequate intervals were allowed between experiments for the salivary iodine to return to normal levels).   The highest value for salivary iodine obtained was 5.675 mgm. per 100 ml.   In two of the three experiments, however, the maximal concentration did not occur until 5 or 6 hours after the ingestion of this compound.

Experiments with sodium tetraiodophenolphthalein.   Two normal subjects ingested a capsule containing 100 mgm. of sodium tetraiodophenolphthalein (55.2 mgm. of iodine).   Iodine in appreciable amounts failed to appear in the saliva for as long as 72 hours after the ingestion of the dye.

COMMENT.   The discrepancy between the present results and those of Heath and Fullerton (9) on the duration of the increased salivary iodine after the ingestion of potassium iodide may be ascribed to the more accurate analytical procedure used in this investigation.   It will be recalled that Heath and Fullerton used a spot test (the addition of ferric chloride and starch to saliva and noting the appearance of a blue color at the end of 20 min.) and observed that in almost all their subjects, iodine could not be detected in the saliva 20 hours after the ingestion of potassium iodide.   Our results show that more than normal quantities of iodine may still be secreted in this fluid as long as 48 hours and occasionally 72 hours after the oral administration of an equivalent amount of iodide. This preferential secretion of iodine by the salivary glands sets up an internal circulation ("inneren Kreislaufs") (1)) since the iodine of swallowed saliva is absorbed from the intestinal tract and again finds its way into the saliva by way of the blood stream.

The salivary glands are impermeable to iodine administered as sodium tetraiodophenolphthalein.   Assuming that these glands cannot secrete iodine in organic combination because of the increased molecular size of such compounds (2, 3), it would appear that tetraiodophenolphthalein fails to undergo cleavage in the body at least to the extent of releasing iodine in elemental form or in simple inorganic combination.   In one patient with obstruction of the common bile duct, the saliva contained only 0.02 mgm. of iodine per 100 ml. whereas the whole blood attained a value of 2.10 mgm. per 100 ml. four days after the administration of sodium tetraiodophenolphthalein for gall-bladder visualization.   Of added interest may be cited the observations in a patient with a broncho-biliary fistula.   Two hours after the administration of this dye, the whole blood showed a concentration of 12.78 mgm. per 100 ml. and the saliva (mixed with sputum and bile) contained 19.22 mgm. per 100 ml.   Obviously, the liver in sharp contrast to the salivary glands is able to secrete iodine compounds of appreciable molecular size.

On the other hand, some organic compounds of iodine may undergo fairly

rapid cleavage in the body. One may infer that the high salivary iodines noted after the ingestion of ethyl diiodobrassidate represents the release of iodine from the iodine-fatty acid complex, presumably by the action of the intestinal lipases. The delayed maximal concentration of salivary iodine after the ingestion of ethyl diiodobrassidate would favor this concept.

CONCLUSIONS

1. Marked increases in the concentration of iodine in the saliva were noted in man after the oral administration of potassium iodide, iodine dissolved in poppy seed oil and ethyl diiodobrassidate.

2. The maximal concentration of salivary iodine occurs in approximately 1 to $2\frac{1}{2}$ hours after the ingestion of potassium iodide or iodine dissolved in poppy seed oil but may be delayed for 5 or 6 hours after the oral administration of ethyl diiodobrassidate. More than normal amounts of iodine in the saliva may still be detected as long as 72 hours after the ingestion of these substances.

3. After the intravenous administration of sodium iodide, the maximal concentration of iodine in the saliva occurs within 5 minutes.

4. The iodine content of the saliva fails to increase after the oral administration of sodium tetraiodophenolphthalein.

REFERENCES

(1) LIPSCHITZ, W. Klin. Wchnschr. 8: 116, 1929.
(2) BARKAN, G. AND W. LEISTNER. Klin. Wchnschr. 8: 117, 1929.
(3) LIPSCHITZ, W. Klin. Wchnschr. 9: 642, 1930.
(4) SCHNEIDER, E. AND E. WIDMANN. Deutsch. Ztschr. f. Chir. 231: 305, 1931.
(5) SCHEFFER, L. Biochem. Ztschr. 259: 11, 1933.
(6) BRUGER, M., J. W. HINTON AND W. G. LOUGH. J. Lab. and Clin. Med. 26: 1942, 1941.
(7) FLEXNER, J., M. BRUGER AND S. MEMBER. Federation Proc. 1 (Part II): 109, 1942.
(8) THIENES, C. H. AND A. J. HOCKETT. J. Lab. and Clin. Med. 16: 843, 1931.
(9) HEATH, C. W. AND H. W. FULLERTON. J. Clin. Investigation 14: 475, 1935.
(10) TREVORROW, V. AND G. J. FASHENA. J. Biol. Chem. 110: 29, 1935.
(11) FASHENA, G. J. AND V. TREVORROW. J. Biol. Chem. 114: 351, 1936.

ARTERIAL AND CENTRAL VENOUS PRESSURE CHANGES FOLLOWING COMPLETE TRANSECTION OF THE SPINAL CORD (C8–T1)

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It is commonly accepted that arterioles and perhaps capillaries are maintained in a partially constricted state by vasotonic impulses which emanate from supra-segmental centers. The removal of this central influence and the consequent diminution of peripheral resistance and augmentation of the capacity of the peripheral vascular bed are employed to explain the initial state of hypotension which develops abruptly after division of the spinal cord at the 8th cervical level. The question has recently been raised whether the importance of normal vaso-tonic control is not greatly over-emphasized (1). From the evidence submitted by the latter investigators, it would appear more exact to attribute the hypotonic response in the spinal animal to a *passive* post-arteriolar dilatation of capillaries, venules and veins in consequence of the withdrawal of their extra-vascular skeletal muscle support. It is presumed that the capacity of these elastic venous structures is primarily regulated by skeletal muscle tone. In accordance with this interpretation, spinal section should lead to reduced skeletal muscle tone, passive dilatation and engorgement of capillaries, veins and venules, reduced venous return and right atrial pressure, impairment of ventricular filling and finally diminution of the stroke volume and thus a lowering of arterial pressure. It becomes a question then as to whether vascular or extra-vascular factors are the primary precipitants of this hypotensive response.

The following studies were conducted to ascertain the changes in *a*, heart rate; *b*, atrial and effective venous pressures; *c*, arterial pulse pressure; *d*, intrathoracic pressure, and *e*, the form of central arterial pressure pulses which are associated with the development of arterial hypotension in the acute spinal animal.

PROCEDURE. This study was conducted on 12 cats. To ascertain whether the removal of extravascular support by rendering the skeletal musculature flaccid is an important feature in the hypotension which develops in the supine spinal animal it was necessary to maintain essentially normal muscle tone for control observations. Hence, these cats were lightly anesthetized with pento-barbital so as to reduce sensory perception without seriously impairing skeletal muscle tone. The doses employed varied from 25 to 28 mgm. of pentobarbital per kilo of body weight.

Operative procedures entailed preparation for simultaneous optical registra-

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tion of the central arterial (carotid), the atrial and intrapleural pressure curves. Arrangements were made to measure mean carotid pressures from a critically damped mercury manometer at regular intervals. A thin-walled stainless steel sound (bore 2.5 mm.) was employed for transmission of atrial pressure fluctuations to a sensitive optical membrane manometer. It was introduced via the right jugular vein until its tip rested either just inside the right atrium or at the junction of this chamber with the superior vena cava. It was also rendered possible to read atrial pressure fluctuations as transmitted to a saline manometer at desired intervals. Intrapleural pressure changes were transmitted through a trocar fixed in the chest wall to another optical membrane manometer. Heparin was used systemically to prevent clotting in the atrial sound, thus eliminating the bizarre and unreliable atrial pressure curves which occur as a result of partial occlusion of the transmission system.

Atrial pressures fluctuate almost continuously throughout the cardiac cycle. What point on the curve, then, represents the pressure most effective for the regulation of ventricular filling? One group of investigators have recently found that measurement of pressures at the peak of the *a* wave yielded the most consistent results in dogs (2). This method of measurement is unsatisfactory in cats, particularly when tachycardia is present, because the *a* wave is frequently masked or entirely absent. We have adopted the expedient of measuring the pressure prevailing just prior to the sudden decline of the V-wave of the venous pulse. Such a procedure seems advisable since this point is usually easily detected. It also conforms with the concept that the major portion of ventricular filling (rapid inflow phase) is governed by the "effective venous pressure" near the end of the isometric relaxation phase. This latter pressure, as defined by Henderson and Barringer, is the algebraic difference between atrial and intrathoracic pressure just prior to the opening of the A-V valves (3). Since there is a cyclic variation in these pressures coinciding with the respiratory cycles, it seemed advisable to measure atrial pressure only from those pulses which coincided with expiratory apnea and with the peak of inspiration. Since the depth of inspiration also varies from moment to moment, it was decided to utilize for discussion purposes only those atrial pressures measured during expiration. The latter remained relatively constant during control observations.

A comparison of atrial pressure values obtained from precisely measured atrial pressure curves with the rough estimates derived from reading rapid saline manometer excursions convinced us that the latter values are very unsatisfactory. Expiratory values from the saline manometer were consistently too low. Inspiratory values were usually too high, though they occasionally equalled or even fell considerably below pressures measured from optical records. It has been our experience that saline manometer readings of atrial pressure cannot be relied upon to indicate either the direction or magnitude of a trend unless atrial pressure changes are extremely severe. In the latter instance, the accuracy of measurement is still unsatisfactory.

The experimental procedure was as follows: After circulatory equilibrium had been established following all preparatory procedures, control records and pres-

sure readings were taken at five minute intervals for 25–30 minutes. The spinal cord was then transected by a method, devised by one of us, which eliminates the necessity of tissue exposure and laminectomy (4). The transection was always at the 8th cervical level. This procedure required from three to five minutes in different animals. Observations were then continued at regular intervals for one hour. At the end of this period, animals were sacrificed and the extent of the cord lesion determined. Those cords not cleanly severed were preserved and examined histologically at some later date.

RESULTS. (a) *Control period.* Skeletal muscle tone in these animals appeared to be reasonably normal. Attempts to passively flex and extend the extremities were opposed by considerable resistance. Spontaneous movement of the limbs was occasionally observed. In most of the animals, control respiration was eupneic in character. Cats 10 and 11 were hyperpneic and bordering on dyspnea, due it is believed to the incidence of pneumothorax incurred while or after inserting the intrapleural trocar. Hence, control atrial, intrapleural and effective venous pressures in these animals were abnormal and are not included in the data presented in this paper. It is noteworthy that these bizarre respirations did not alter the arterial blood pressure picture significantly with the result that the arterial pressure changes in these animals are included with the others. At this stage of the experiment, the nictitating membranes were partially relaxed, a common observation in cats under light pentobarbital anesthesia.

To conserve space, fairly representative results from only one of our 12 experiments are presented in table 1. The average trend of the fairly uniform events is depicted in graphic form in figure 1. In the latter, the direction, if not always the magnitude, of individual responses is accurately represented. A perspective of the average control values is presented in tabular form below:

MM. OF HG				MM. OF SALINE					
				IAP		IPP		EVP	
H.R.	S.P.	D.P.	M.B.P.	INS.	EXP.	INSP.	EXP.	INSP.	EXP.
190	154	109	129	−30	+32	−81	−26	+51	+58

Arterial pressures were well within the range of normalcy for pentobarbitalized cats. The atrial and effective venous pressures compare favorably with those previously reported as normal for barbitalized dogs (5).

(b) *Spinal transection period.* With this blind spinal puncture technique the operations were completed within three to five minutes. During a portion of the procedure, as in other methods, the spinal cord was mechanically irritated. As a result, arterial, atrial and effective venous pressures were abruptly elevated. Arterial pressures well above 200 mm. of Hg were observed in every animal. The systolic pressure was elevated to a much greater extent than the diastolic pressure, hence pulse pressures were greatly augmented. The heart rate was moderately retarded and on a few occasions momentary cardiac irregularities developed.

(c) *Primary post-operative phase.* This phase is arbitrarily limited to the

period required for arterial blood pressure equilibrium to be regained. It varied from 10–20 minutes in the different animals. During this time, arterial and atrial pressures descended from their artificially elevated levels, the former more
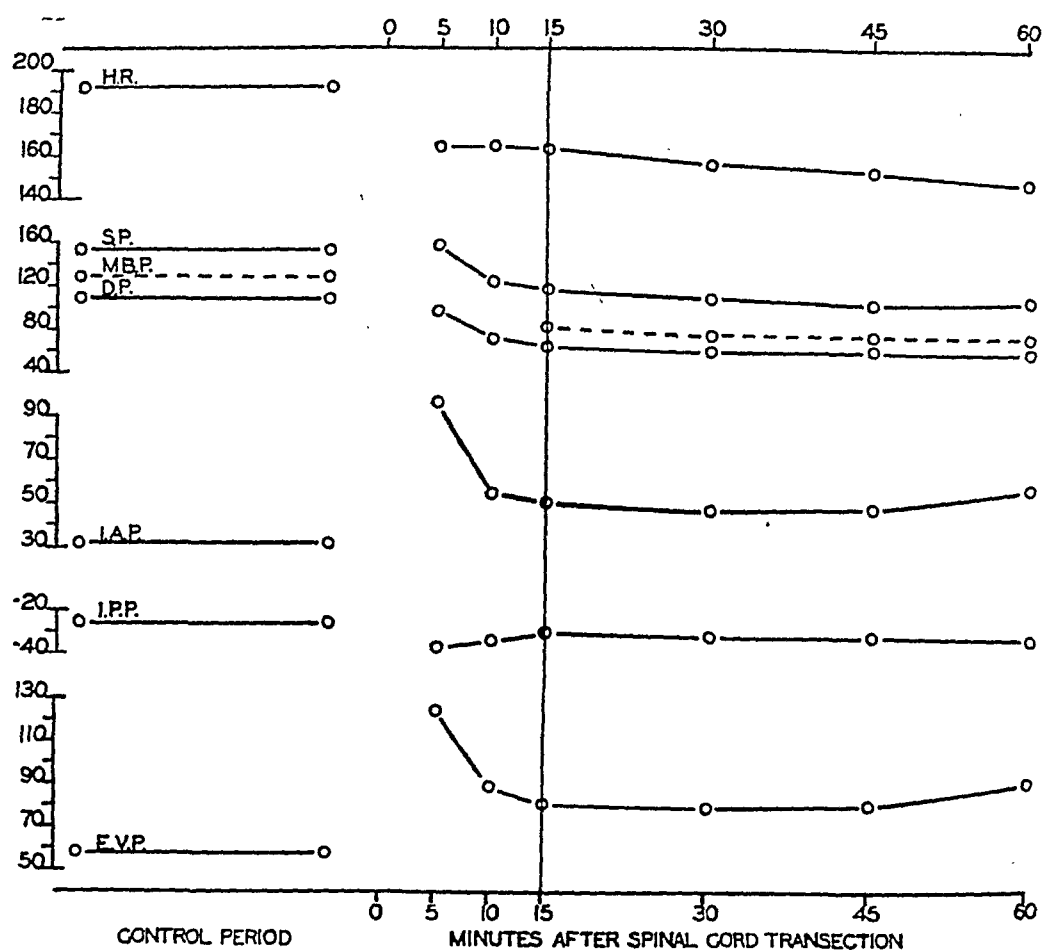
Fig. 1. Average responses of 12 cats whose spinal cord was transected at the 8th cervical level. *H.R.*, heart rate; *S.P.*, systolic pressure; *M.B.P.*, mean blood pressure; *D.P.*, diastolic pressure; *I.A.P.*, intra-atrial pressure; *I.P.P.*, intrapleural pressure, and *E.V.P.*, effective venous pressure.

TABLE 1*

EXPERIMENT NO. 4	CONTROL VALUES	CHANGES AFTER TRANSECTION					
		5'	10'	15'	30'	45'	60'
Heart rate (beats per minute).........	153	120	138	135	130		
Systolic pressure (mm. of Hg).........	170	188	152	139	130	135	125
Diastolic pressure (mm. of Hg)........	120	120	98	92	82	89	85
Pulse pressure (mm. of Hg)............	50	68	54	47	48	46	40
Mean blood pressure (mm. of Hg)	134			98	80	94	90
Atrial pressure (mm. of saline)	35	40	30	40	60	70	60
Intrapleural pressure (mm. of saline)..	−25	−50	−40	−10	−35	−33	−35
Effective venous pressure (mm. of saline).............................	60	90	70	50	95	103	95

* A copy of similarly tabulated results for all 12 experiments will be furnished on request.

rapidly than the latter. By the end of the *primary phase* arterial pressures were fairly well stabilized at subcontrol levels. The pulse pressures rapidly approached or assumed preoperative values. The form and slopes of the arterial pressure pulses were likewise unchanged generally. In cats 7 and 12, however, bizarre pulses developed which made the accurate measurement of pulse pressures impossible. As will be mentioned later, these two animals were probably in a state of irreversible circulatory shock.

Atrial pressures descended slowly. By the end of the primary phase, they were again approaching stabilization at supra-control levels. In no instance did they fall significantly below their respective control levels. A similar course was followed by effective venous pressures. Intra-pleural pressures of the expiratory cycles were not appreciably altered by cord transection. The changes in the rate of respiration were neither of significant magnitude nor uniform in direction.

(d) *Secondary post-operative phase.* This constitutes the remainder of the hour of post-operative observation. It varied from 40–50 minutes in duration. This was a period of minor changes with little apparent significance in this problem. Arterial pressures had not begun to approach control values by the end of this period. There are reasons for speculating that arterial pressure recovery was delayed by the use of a non-volatile anesthetic. When similar hypotensive responses were elicited in etherized or decerebrate cats, the degree of recovery was quite striking in many animals within the hour following spinal transection (6).

(e) *Miscellaneous post-operative findings.* Skeletal muscles innervated behind the cord lesion were deprived of their pre-operative tone early in the *primary phase*, prior to the decline of arterial blood pressure. The hind limbs in particular displayed a complete flaccidity. Preoperative tone in skeletal muscles innervated above the lesion was either retained or augmented as the experiments progressed. Respiration, originally costodiaphragmatic, was rendered purely diaphragmatic after cord section. In spite of this, intrapleural pressure relationships were not disturbed. It appears that diaphragmatic movements are alone capable of sustaining adequate pulmonary ventilation in the cat under these conditions. The nictitating membranes were completely relaxed after transection.

Gross and histological examination of the injured cords yielded anatomical evidence that bilateral division of the spinal cord was complete in all save animal 8. The latter appears to have undergone a right hemisection of the cord, even though its functional manifestations were similar to those observed in the other cats.

DISCUSSION. Arterial pressures in these lightly anesthetized supine cats began to decline abruptly after spinal cord transection at the 8th cervical level. Circulatory equilibrium was again re-established at hypotensive levels within 10–20 minutes (*primary phase*). In all save two animals (7 and 12), these subcontrol arterial pressures exceeded 70 mm. of Hg. There was no evidence that these animals suffered the condition of circulatory shock. Their pressure pulse

contours were essentially those observed during the control periods. It seems quite likely that these cats would have survived if the period of observation had been considerably extended. The situation in animals 7 and 12 was complicated by immeasurable hemorrhages which were associated with the operative procedures. In these, arterial pressures dipped to "critical levels." Arterial pressure pulses revealed "high systolic peaks" with little or no diastolic gradients, a type commonly encountered in hemorrhagic and shock-like conditions. It is suspected that these two animals would have become fatalities if the period of observation had been prolonged. Circulatory shock apparently does not occur as a result of spinal cord transection when mechanical trauma and hemorrhage are curtailed to a minimum.

The question arises as to which of the mechanisms involved in the maintenance of normal arterial blood pressure are eliminated or impaired in the animal rendered acutely spinal. It is believed that the relative importance of cardiovascular as compared with extra-vascular mechanisms in sustaining normal pressures in the supine animal becomes evident after the various cardiodynamic events associated with the decline of arterial pressure during the *primary phase* are analyzed on the basis of established fundamental hemodynamic concepts. It is judged, a priori, that the hypotensive response to spinal transection was a manifestation of one or more of the following dynamic events; *1*, a diminution of arterial resistance, *2*, a retardation of heart rate, *3*, an impairment of ventricular stroke volume, and *4*, an increase in the capacity of the system of Windkessel structures.

Under controlled experimental conditions, events *1* and *2* favor a predominant lowering of diastolic pressure and hence an augmentation of pulse pressure. On the other hand, events *3* and *4* favor a diminution of the pulse pressure since systolic pressures are predominantly lowered. Any one of these events acting singly would alter pulse pressures significantly. It appears that several of these events were involved in a reaction in which systolic and diastolic pressures were reduced to approximately the same extent.

Hemodynamically, the major portion of the diastolic pressure decline must be attributed to dynamic changes *1*, *2* or both. Slowing of the heart rate appears to be rather inconsequential in this situation. Despite moderate retardation, heart rates remained markedly tachycardic. The thought occurs that the moderate slowing may have tended to prevent a greater reduction of arterial pressure through the expedient of permitting more adequate ventricular filling at each stroke, thus improving the systolic discharge. It is also noteworthy that equivalent hypotensions developed in two animals in which heart rates were slightly accelerated after spinal transection. This leads to the belief that the major portion of the decline in diastolic pressure is primarily the result of dilatation of peripheral arterioles due to the interruption of vasotonic impulses which normally maintain arterioles in a partially constricted state. This is the usual explanation of the decline in blood pressure in the spinal animal. It is obvious, however, that this event alone cannot explain the equivalent decrease in systolic pressure.

Recent observations by H. Smith and his collaborators might seem to be pertinent in this respect (1). The inference is drawn from their work that the elastic veins and perhaps capillaries are deprived of their normal extravascular skeletal muscle support after transection of the cord and that the capacity of the great venous reservoir is passively augmented. As a result, it is assumed that there is considerable pooling of blood in the peripheral venous system with a consequent reduction of atrial and ventricular filling pressure which must lead to a reduction of systolic discharge. At least a partial answer to this suggestion that a reduced systolic discharge is involved in this systolic pressure decline is obtained from the observations that atrial and effective venous pressures, if altered at all, were slightly elevated during the *primary phase,* while the state of hypotension was developing. Furthermore, the slopes of the ascending limbs of arterial pressure pulses were not reduced as would be anticipated if the ventricle were beating hypodynamically because of inadequate filling. Therefore the atrial pressures and ventricular stroke volumes in these supine animals do not appear to have suffered when the skeletal musculature of the thorax, abdomen and hind extremities were abruptly rendered completely flaccid. It is quite reasonable however to assume that this factor would assume major importance if the animals' position were altered such that it was necessary to counteract the effects of gravity on the venous return.

So far as these supine animals are concerned, there remains by elimination the probability that factor *4* (increased capacity of aorta and large arterial branches) is primarily responsible for the major reduction of systolic pressure. Unfortunately, little is known at present of the physiological characteristics of the Windkessel system. There is evidence, however, that the diameter of the aorta changes markedly in response to reflex and humoral stimulation (7). It is not unreasonable, then, to assume that the muscular elements of the Windkessel system, like the smaller peripheral vessels, are normally subject to vasotonic impulses which maintain the large vessels in a partially constricted state. The capacity of these vessels would thus increase when the regulatory influences were removed by spinal transection. This then may be the mechanism which accounts for the fall in systolic pressure not accounted for by the arteriolar dilatation.

SUMMARY AND CONCLUSIONS

This study concerns the acute arterial hypotension and associated phenomena which occur following transection of the spinal cord (C8) of supine lightly anesthetized cats. When the transection procedures were uncomplicated by severe hemorrhage, pressures did not descend to critical levels. The hypotension developed after the skeletal musculature of the thorax, abdomen and hind limbs, which supposedly support the elastic post-arterial vessels, had been rendered completely flaccid. Apparently, however, the venous return of these supine animals was not impaired by the loss of this extravascular support. The criteria for this concept are based upon the fact that atrial and effective venous pressures were, if anything, slightly elevated, while the heart rates remained

tachycardic. The slopes of the ascending limbs of arterial pressure pulses revealed no changes indicative of an impaired stroke output.

In explaining the equivalent decrease of systolic and diastolic pressures on a hemodynamic basis, it was deduced that reduction of peripheral resistance is a major contributing factor, but that some additional dynamic event must be involved. The analysis suggests that an increase in the capacity of the aorta and its immediate large branches is involved. The acute hypotension following spinal transection may be considered to result from an increase in the capacity of and a reduction in resistance to blood flow throughout the entire arterial system from the root of the aorta to the capillaries. There is no evidence to support the postulate that an impairment of skeletal muscle tone or the extravascular mechanism is herein concerned with the fall in blood pressure in these supine animals.

REFERENCES

(1) SMITH, H., E. A. ROVENSTINE, W. GOLDRING, H. CHASIS AND H. A. RANGES. J. Clin. Investigation 18: 319, 1939.
(2) WERLE, J. M., R. S. COSBY AND C. J. WIGGERS. This Journal 136: 401, 1942.
(3) HENDERSON, Y. AND T. B. BARRINGER, JR. This Journal 31: 288, 352, 1913.
(4) TREAT, A. E. This Journal 33: 310, 1941.
(5) WIGGERS, C. J. This Journal 33: 13, 1914.
(6) WIGGERS, H. C. Unpublished observations.
(7) WIGGERS, C. J. This Journal 124: 603, 1938.

THE VOLUME FLOW OF RESTING SALIVARY SECRETION

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The term "resting salivary secretion" is used here to mean the salivary secretion which occurs in the absence of any obvious external salivary stimulus. A few investigators (Becks, 1939; White and Bunting, 1936) have previously used this term; and they, and others (Zaus and Fosdick, 1934), have given quantitative data on the volume of this secretion. The data of these authors are either too few or are published in such a form that it is difficult to determine the accuracy with which this measurement or a mean of several such measurements on an individual can be reproduced; in fact, the assumption that the volume of resting salivary flow is a characteristic of an individual could possibly be challenged.

The present data, collected on male students of two classes of sophomore students of dentistry aged 20 to 29 years, are analyzed in relation to this problem in an attempt to contribute to the formulation of a standard technic, and to the establishment of normal limits, for the volume flow of resting salivary secretion.

METHOD. All determinations are carried out in mid-morning or mid-afternoon to avoid as far as possible any effect of meals or hunger on salivary flow. The subjects are requested not to smoke during the ten minutes prior to the determination; they are warned against gum chewing or candy eating previous to the determination. Psychic influences, such as resentment, embarrassment, etc., are avoided. The subject, in a sitting position, bends his head forward to the horizontal and allows the saliva to flow into a 50 cc. beaker placed lightly in contact with the lips, under the opened mouth. Mouth breathing, movement of the tongue, swallowing movements, etc., are avoided. The saliva drains into this beaker for 5 minutes in order to allow time for the establishment of a steady flow, so that the subsequent volume outflow of saliva represents the volume secreted. This beaker is now removed with a sliding motion to take the excess saliva from the lips and a second 50 cc. beaker is quickly substituted. After exactly 5 minutes, the second beaker is removed in the same manner as the first, and the volume is measured in a 10 cc. graduated cylinder after adding 0.1 cc. butyl alcohol to destroy the foam. No correction is made for the volume of alcohol added, since this approximately equals the volume of saliva left in the beaker.

The method used is a rather arbitrary one based to a great extent on a quantity of data obtained with one subject and on the known facts of salivary flow. This general method of measuring total salivary flow has been used by others (Becks, 1939; White and Bunting, 1936). In our use of this method, precautions were taken to avoid stimulation of salivary flow by the passage of air through the mouth by having the collecting vessel (beaker) in close contact with the lips. The time period of salivary collection used results from a compromise

between the obtainment of a volume sufficient for reasonably accurate measurement and the avoidance óf restlessness of the subject. The reasons for most of the precautions taken before and during the period of saliva collection are obvious. Some of these precautions are difficult to execute with certainty, since many of these depend upon the co-operation of the subject. This element of uncertainty can be reduced by direct observation of the subject before and during the determination. This was done for two determinations on each of 37 subjects; the values obtained did not differ greatly from those obtained by the subject himself after suitable instruction; however, it is possible that in certain

TABLE 1

Values for the volume of resting salivary flow obtained on 10 subjects (S)

Each figure represents the number of cubic centimeters of saliva collected in a 5 minute period. The values are recorded in the order in which the determinations (D) were made. The mean (M), the standard deviation (S.D.), the standard deviation in per cent (S.D. per cent), and the standard error of the mean (S.E.) are given for each subject.

S \ D	W. A.	A. D.	I. G.	M. G.	F. J.	G. L.	G. Q.	R. R.	D. S.	C. W.
1*	1.4	3.0	3.0	1.1	0.3	0.5	0.8	3.6	3.3	1.1
2*	0.4	2.3	4.8	1.0	0.5	0.9	1.0	1.7	2.2	1.6
3	0.6	2.1	2.2	1.3	0.9	0.5	1.5	3.1	3.3	2.4
4	1.3	2.8	1.6	2.5	0.4	0.5	1.2	2.7	2.9	1.7
5	1.5	2.1	1.6	1.2	1.1	0.8	1.0	3.6	2.6	1.3
6	1.0	2.0	2.1	1.9	1.6	0.4	1.2	2.9	2.9	1.7
7	0.7	2.1	2.4	1.6	0.4	1.2	1.3	2.1	2.6	1.8
8	1.2	2.2	3.1	1.5	1.2	0.9	1.0	4.6	2.6	2.0
9	1.8	2.5	1.3	1.6	1.0	0.9	1.2	2.1	2.0	2.4
10	0.7	2.1	2.8	1.7	1.4	1.0	1.2	2.9	1.9	1.8
11	0.7	1.1	2.2	2.1	1.0	1.3	1.0	1.8	2.2	1.6
12	0.9	2.2	3.0	2.1	1.6	0.9	1.5	4.5	1.8	1.1
13	0.9	2.2	2.2			0.5	0.8	2.6	2.7	
14		2.0	1.9			0.5	0.3	2.5		
M..........	1.01	2.19	2.44	1.63	0.95	0.77	1.07	2.91	2.54	1.71
S.D........	0.41	0.43	0.88	0.45	0.47	0.29	0.30	0.91	0.49	.43
S.D. (%)....	40	20	36	28	49	38	28	31	19	25
S.E........	0.11	0.11	0.23	0.13	0.14	0.0S	0.0S	0.24	0.14	0.12

* These values were taken under the direct supervision of a trained observer.

instances the psychic effect of this direct supervision may have decreased the salivary flow in certain subjects (see table 1). Because of the possibility of the psychic factor influencing the results, most of the data was obtained by the individual subject with a minimum of supervision.

RESULTS. Table 1 gives data obtained on a series of 10 of the subjects for the purpose of estimating the variability of values of resting salivary secretion in each individual subject. This group represents approximately one-quarter of the total number in one sophomore class, and so forms a large sample of this class. This sample was probably not greatly distorted by the method of selec-

tion; for it represents the first 10 students to volunteer as paid subjects. The first three values on these subjects were obtained in the summer and fall and the remainder in the winter of the current school year (1942–1943). The table also gives the mean, the standard deviation, the standard deviation expressed as per cent of the mean, and the standard error of the mean of each series of determinations.

Table 2 shows that the distribution of values around the mean value for each subject of table 1 conforms approximately to the theoretical.

Table 3 gives a limited number of single determinations taken a year apart on a few students. These data indicate that the resting salivary flow does not vary to a much different extent over an appreciable time period than it does over a short period of time (see table 1).
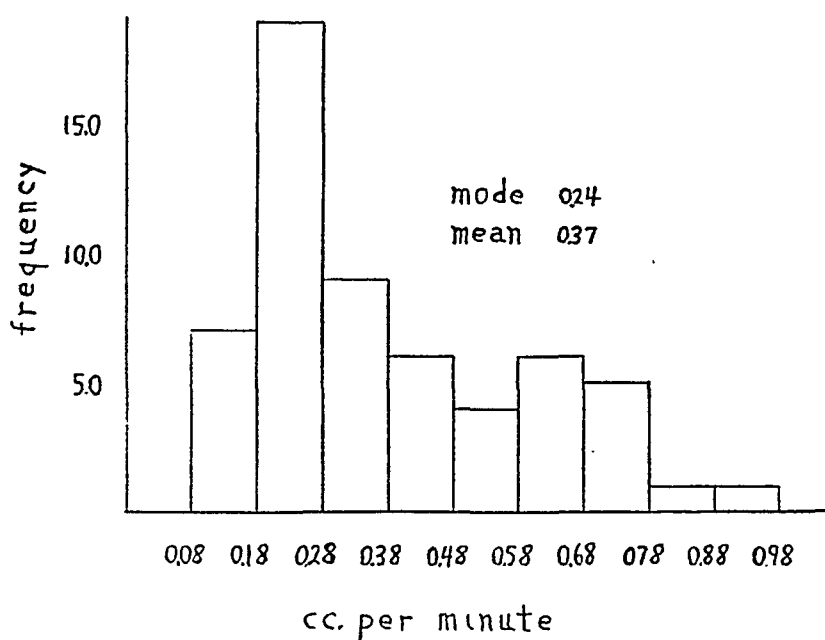
Fig. 1. The distribution of values for the resting flow of salivary secretion obtained on 58 subjects. The values used are the means of two determinations on each subject. The frequency is the number of individuals actually present in each group.

Figure 1 shows the distribution of the means of 2 values of resting salivary secretion of 58 subjects.

DISCUSSION. The variability of values for the volume of resting salivary secretion in the individual subject is quite large (see table 1). From our experience, it is believed that this variability could not be greatly decreased by controlling the experimental conditions more accurately; and, for the present, it is assumed that a rather large variability is inherent in the flow of saliva and that the values obtained represent random samples of the salivary output for the given set of conditions. This assumption is supported by the fact that the standard deviations, when expressed in per cent, are of the same order of magnitude in the 10 subjects studied (see table 1), and the fact that values for each subject are distributed around the mean value in fair agreement with the theoret-

ical distribution (see table· 2). Also supporting this is the fact that others (Becks, 1939; Zaus and Fosdick, 1934) have reported considerable fluctuation in the rate of saliva flow in the individual.

Granting this assumption, it is seen that the mean value of salivary flow for a given subject may be determined quite accurately if a sufficient number of determinations are made (see standard errors of the mean values in table 1). Since the mean volume of salivary secretion is sufficiently different amongst the subjects studied, it may be concluded that the mean volume of salivary flow is a characteristic of the individual subject. A few salivary flow values taken

TABLE 2

The distribution of values obtained on each subject (S) about their mean (from table 1)

The interval (I) width is one standard deviation. All values are in per cent. The theoretical distribution is given in the column at the extreme right. The mean of the percentages (M) found in each interval is given in the next to the last column.

S ⟍ I	W. A.	A. D.	I. C.	M. G.	F. J.	G. L.	G. Q.	R. R.	D. S.	C. W.	MEAN	THEO-RET.
+2 to +3	0	0	7	0	0	0	0	0	0	0	1	2
+1 to +2	15	14	0	25	17	14	14	14	15	17	14	14
0 to +1	23	36	29	17	41	43	36	21	46	25	32	34
0 to −1	54	43	57	41	17	36	43	50	15	41	40	34
−1 to −2	8	0	7	17	25	7	0	14	23	17	12	14
−2 to −3	0	7	0	0	0	0	7	0	0	0	1	2

TABLE 3

Values for the volume of resting salivary flow in 5 subjects taken at different times

Each figure represents the number of cubic centimeters of saliva collected in a single 5 minute period.

SUBJECT	FALL 1941	SPRING 1942	SUMMER 1942	FALL 1942
H. H.	1.8	1.8	1.7	1.9
G. L.	0.5	0.9	0.5	0.5
H. R.	2.4	3.2	3.4	2.3
S. S.	1.1	1.5	1.8	1.7
W. W.	3.1		2.9	3.0

over a period of a year on a limited number of subjects give support to this conclusion (table 3).

Some indication of the number of determinations that should be made on a subject in order to know with some accuracy his mean resting salivary flow can be obtained from the data of table 1 by calculating the standard error of the mean from the standard deviation for various numbers of determinations used to obtain the mean by use of the formula S.E. $= \dfrac{S.D.}{\sqrt{N}}$. As is well known, the standard error decreases rapidly at first, and then more slowly, as the value of N is increased. Thus it might be expected that if only 4 measurements were taken on each of these 10 individuals, the standard errors would have values of the

magnitude of 10 to 24 per cent of their mean values; while if 9 measurements were made the standard errors would have values of the magnitude of 6 to 17 per cent of their mean values. Conclusions from such a calculation should be applied with some caution however; for it should be realized that the fewer values taken, the greater will be the error due to a single erratic determination. Also greater variability may be found in some subjects than occurs in these individuals who were studied.

The distribution of the means of 2 values for 58 subjects is shown in figure 1. Although the mean of 2 values is certainly an inaccurate measure of the salivary flow of a subject, it is likely that this distribution curve represents in a general way the true distribution of values for these subjects. It is seen that the curve is skewed, the mean and the mode of the distribution curve being respectively 0.37 and 0.24 cc. per minute. The standard deviation was not calculated because of the skewness of the distribution. Resting salivary flow values obtained by the authors mentioned earlier are somewhat higher than the present values. Differences in technic, in experimental conditions, and in type of subject may be responsible for this.

SUMMARY

Using the method described for determining the resting volume of salivary secretion, evidence was obtained that the mean of a sufficient number of determinations represents a value which is reproducible and characteristic of the individual. The distribution of the mean of 2 values of a limited number of subjects is skewed. The modal value is about 0.24 cc. per minute. The lowest mean of two values obtained was 0.08 cc. per minute; and the highest, 0.88 cc. per minute.

REFERENCES

BECKS, H. J. Dent. Res. **18**: 431, 1939.
WHITE, J. AND R. W. BUNTING. This Journal **117**: 529, 1936.
ZAUS, E. A. AND L. S. FOSDICK. J. Dent. Res. **14**: 3, 1934.

DEVELOPMENT OF HOMEOTHERMY IN SUCKLING RATS

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Perhaps the most useful quantitative index of postnatal *structural* development is increase in size. Among the more interesting indices of *functional* development is increase in what Cannon terms homeostasis (1), the maintenance of a constantly favorable internal environment in the face of external change.

It is generally known that human infants, and young mammals of many other species, do not possess the ability to maintain a constant body temperature; their homeothermic mechanisms are not well developed (2). As Cannon has remarked, homeothermic mechanisms are not required by the developing mammal before birth. At birth, however, survival becomes increasingly contingent upon the exercise of these mechanisms. This report traces the development of homeothermy in the albino rat from birth to weaning at 22 days of age.

METHODS. We investigated the development of ten litters of suckling rats totaling 93 animals. Since intervals of two or more days separated repeated observations on any single individual, so as to avoid the possibility of injury or decreased growth rate due to the experimental treatment, each point in figure 1 represents the average of data from about twenty animals. This procedure may have been the cause of some irregularity in the curve. Records were obtained by placing each subject in a covered container immersed in water at a temperature of 14.7–15.0°C. for fifteen minutes, and then measuring (by means of a thermocouple) the difference between the rectal temperature of the animal and the temperature of the water bath.

RESULTS. Figure 1 shows the development of homeothermy in suckling rats. "Homeothermy" is gauged by the difference between rectal and environmental temperatures, when the environment is cool. Body weights and heat production (oxygen consumption) are also given on the arithlog grid of figure 1 for purposes of general comparison.

At birth the body temperature was only one degree above that of the environment. At the age of one day, the difference between body and environmental temperatures rose to 3°–4°C., and so on, as shown in figure 1. Under the given conditions normal homeothermy for the species was practically fully achieved at the weaning age, 22 days, when the body temperature remained at 37°C. in an environment of 15°C. It is, perhaps, significant that the attainment of full homeothermy comes at an age when the mother is no longer available as a source of warmth.

The central nervous center for the adaptive reaction to cold appears to be

located in the hypothalamus (3), and the age curve of homeothermy probably reflects in part the functional development of the heat-regulating center of the hypothalamus. Shivering and other muscle reflexes tending to increase the internal temperature become apparent at age nine to twelve days (4). The development of the endocrines, particularly the adrenals (5) and thyroid (6), may play a rôle in the development of homeothermy. Age changes in homeothermy may be related to age changes in energy metabolism. In this respect it is interesting to note that figure 1 shows similar irregularities in the curves of heat production and homeothermy in the region of nine days. Thus, the age curve
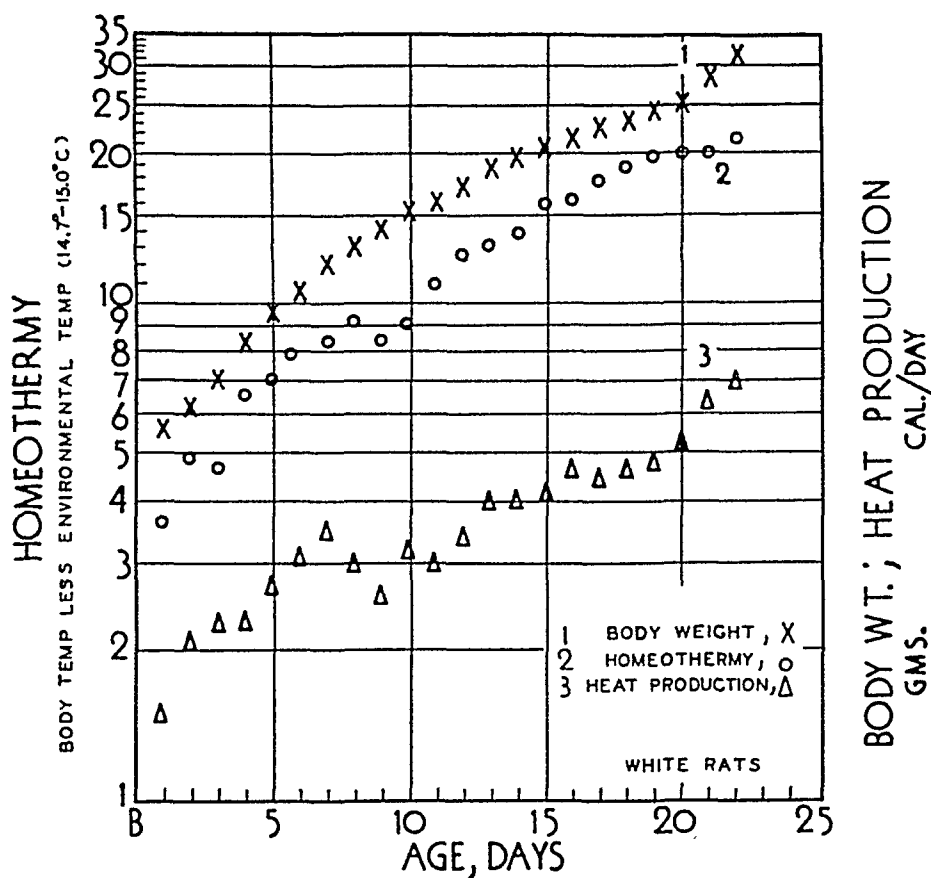
Fig. 1. Age curves of development of homeothermy (curve 2), body weight (curve 1), and heat production (curve 3) in rats.

of homeothermy in figure 1 represents the summation of contributing nervous, vascular, hormonal, and metabolic mechanisms.

SUMMARY

Homeothermy in white rats, as measured by the difference between body and environment temperatures, is practically complete at the weaning age of 22 days. The curves of homeothermy and heat production show similar irregularities in the region of nine days, at which age shivering and other muscular reflexes to cold become apparent.

REFERENCES

(1) Cannon, W. B.   Physiol. Rev. **9**: 397, 1929.
(2) Stier, T. J. B. and G. Pincus.   J. Gen. Physiol. **11**: 349, 1927–28.
   Kleitman, N., S. Titelbaum and H. Hoffman.   This Journal **19**: 48, 1937.
   Pincus, G., G. D. Sterne and E. Enzmann.   Proc. Nat. Acad. Sc. **19**: 729, 1933.
   Romanoff, A. L.   Science **94**: 218, 1941.
(3) Ranson, S. W.   Proc. Assn. Res. Nerv. and Ment. Dis. **20**: 342, 1940.
(4) Gulick, A.   This Journal **76**: 206, 1926; **119**: 322, 1937.
(5) Cannon, W. B. et al.   Idem. **79**: 466, 1926–27.
   Freeman, N. E.   Idem. **113**: 384, 1935.
(6) Ring, G. C.   Idem. **125**: 205, 1939, and others.

THE EFFECT OF MANGANESE AND BORON COMPOUNDS ON THE RAT INCISOR

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The present communication represents a part of a study intended to search for elements which can produce enamel hypoplasia. It was thought that in finding the common qualities of such elements the primary causative factors in the production of enamel hypoplasia could be determined. This study is concerned with the effect of manganese and of boron.

Manganese is present in all living tissues, both plant (1, 2) and animal (3). It is present in the blood in fairly constant concentration (3). Addition of 3.6 per cent manganous chloride ($MnCl_2 \cdot 4H_2O$) to the diet produces moderate to severe rickets (4). At the present time it is the most potent activator of liver phosphatase known (5).

It has been fairly well established that the presence of *boron* is vital for the normal utilization of calcium by plants even when the supply of the latter is adequate (6). Apparently boron is not essential for the normal growth and development of the rat (7).

Since no information is available concerning the effects of manganese and boron on the dental tissues, an investigation into such effects seemed advisable. Special interest was attached to the experiments on the influence of boron following the recent report that a correlation exists between the fluorine and boron contents of natural waters (8).[1]

METHODS AND MATERIAL. The present report is based on studies of the upper incisors of 27 white rats. Thirteen animals were given a single subcutaneous injection of 1 cc. of manganese chloride (C. P.) solution in concentration varying from 1 to 15 per cent (table 1). Eight animals were given a single subcutaneous injection of 1 cc. of a boron containing compound (table 2). Six animals served as controls.

Albino rats used in these experiments were bred Wistar Institute stock and fed dog chow (Purina). Twice a week, lettuce, carrots and dry bread crusts were added to the diet. The animals were kept in a light room—3 to 5 in a cage. The animals were killed by prolonged narcosis with chloroform. Four animals receiving the highest dosage of manganese chloride and one animal injected with a solution saturated with both borax and boric acid died before the end of the experiment (tables 1 and 2).

[1] After the paper had been submitted to the publisher, H. T. Dean, U. S. Public Health Service, kindly called attention to the fact that "apparently there is little consistency in this relationship when viewed over a broad expanse, such as the country as a whole, although in circumscribed geographical-geological areas the relationship may hold."

The specimens were fixed immediately in Zenker-formalin (Maximow) for one day or Bouin's solution for two days. The dissected jaws were decalcified in

TABLE 1

Experimental data and changes of enamel and dentin of 13 rats injected with 1 cc. of manganese chloride solution

NO. AND SEX	AGE AT START	WEIGHT AT START	WEIGHT AT END	CONC. OF MnCl₂ SOLUTION	pH	DOSAGE IN MGM. PER KGM.	LENGTH OF EXPER.	ENAMEL HYPO-PLASIA	INCREMENTAL LINES	
									Enamel	Dentin
		gm.	gm.	per cent			days			
1 ♂	5 m. 6 d.	235	239	1.0	4.30	42	8	—	—	—
2 ♂	5 m. 20 d.	260	248	2.5	4.20	96	8	—	—	+
3 ♀	2.5 m.	139		2.5	4.20	181	6	+	—	+
4 ♂	5 m. 20 d.	234	216	5.0	4.08	213	8	+	+	+
5 ♀	2.5 m.	136		5.5	3.95	404	6	+	+	+
6 ♂	6 m. 9 d.	237	254	10.0	3.95	417	8	+	±	+
7 ♀	2.5 m.	139		8.0	3.95	576	6	+	+	+
8 ♂	6 m. 9 d.	230	239	15.0	3.76	652	8	—	—	+
9 ♀	2 m.	100		8.0	3.95	808*	6	+	—	+
10 ♀	2 m.	99		8.0	3.95	808	6	+	+	+
11 ♂	2 m.	115		11.0	3.87	956*	1	—	—	—
12 ♀	2 m.	113		11.0	3.87	973*	4	+	—	+
13 ♀	2 m.	106		15.0	3.76	1429*	1	—	—	—

* Indicates that the animals died spontaneously; the others were sacrificed by over anesthesia with chloroform.

TABLE 2

Experimental data and changes in dentin and enamel of 8 rats injected with 1 cc. of boron compounds

NO. AND SEX	AGE AT BEGIN.	WEIGHT		INJECTION OF 1 CC.	pH	BORON PER KILO	DURA-TION	HYPO-PLASIA	INCREMENTAL LINES	
		At begin.	At end						Enamel	Dentin
	months	grams	grams			mgm.	days			
14 ♂	1	48	71	5% borax	8.9	39.6	9	—	+	+
15 ♂	1	53	75	1% borax & 1% bor. acid	8.1	40.1	9	—	—	—
16 ♀	1	47	67	5% borax	8.9	40.4	9	—	—	+
17 ♀	1	41	57	5% borax	8.9	46.3	9	—	—	—
18 ♂	1	43	66	1% borax & 1% bor. acid	8.1	49.3	9	+	—	—
19 ♂	1	53	70	5% borax & 4% bor. acid	8.0	168.0	9	—	+	+
20 ♂	1	48	65	5% borax & 4% bor. acid	8.0	185.0	9	—	+	+
21 ♀	1	45		5% borax & 4% bor. acid	8.0	198.0	3*	Not investigated		

* Indicates that the animal died spontaneously; the others were sacrificed by over anesthesia with chloroform.

5 per cent nitric acid, embedded in celloidin and the sections stained with hematoxylin and eosin.

FINDINGS. *A. Injection of manganese chloride.* Manganese chloride solution produced necrosis at the site of the injection in all instances.

Enamel hypoplasia was found in 8 of 9 rats receiving 180 to 975 mgm. MnCl₂ per kgm. body weight and investigated 4 to 8 days after injection. In all instances the enamel matrix in the area of the hypoplasia is very thin and wedge-shaped with the base of the wedge toward the incisal end. The hypoplastic area is sharply demarcated against the areas of normal enamel at both apical and incisal ends. The length of the hypoplastic zone varied from 0.3 to 0.6 mm. in the eight animals studied.

The enamel epithelium covering the hypoplastic area shows changes of different degrees, which are apparently not related to the length of the experiment or to the dosage. The enamel epithelium might be formed by small cuboid cells (fig. 1, A), or spindle-shaped cells orientated in different directions (fig. 1, B). In figure 1, C, the incisal covering of the hypoplastic area is formed by cuboid
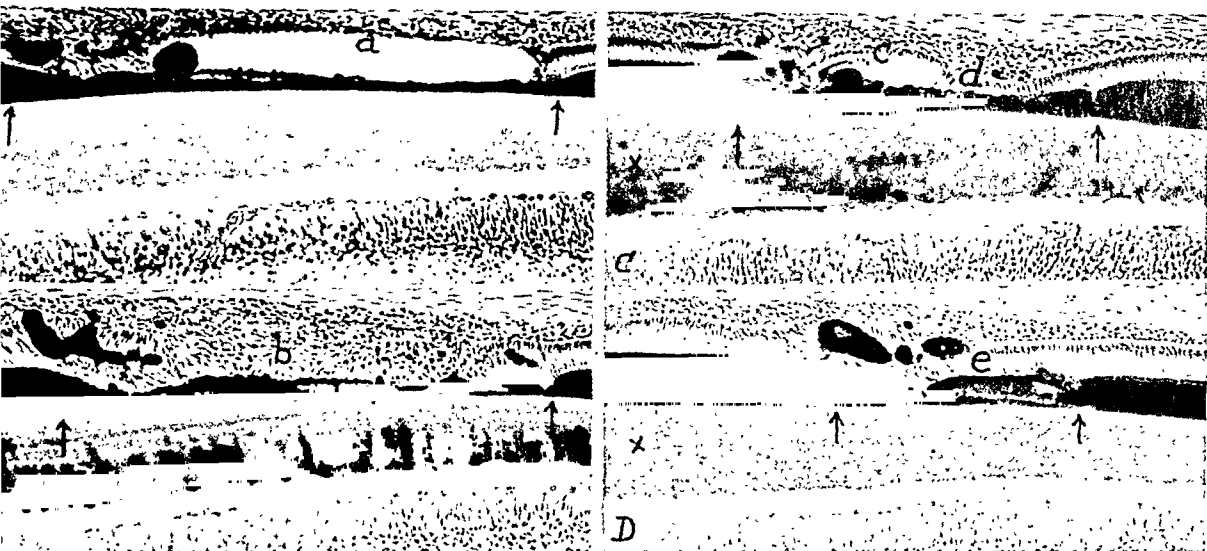

Fig. 1. Single subcutaneous injection of manganese chloride. Variation of changes in the enamel organ covering the enamel hypoplasia (areas between the arrows) and incremental bands in the dentin (*x*) Magnification ×150. The ameloblasts in figure 1A (no. 6) are cuboid (*a*); in figure 1B (no. 10) spindleshaped (*b*); in figure 1C (no. 5) incisally cuboid (*c*) and apically high columnar (*d*); in figure 1D (no. 3) the ameloblasts show minor changes (*e*).

cells, whereas the cells at the apical end are high columnar cells. In figure 1, D, where the hypoplastic area is shorter than in previous cases, most of the ameloblasts covering this area show minor morphological changes.

The photomicrographs demonstrate further differences in regard to the attachment of the enamel epithelium to the hypoplastic area and in regard to the changes of the stratum intermedium and outer enamel epithelium. In figure 1, A and C, the enamel epithelium is detached from the surface of the enamel matrix and the stratum intermedium and outer enamel epithelium can not be distinguished. In the other two cases there is no such detachment and the stratum intermedium and outer enamel epithelium can generally be distinguished (fig. 1, B and D).

In all instances the enamel hypoplasia was covered by an amorphous mass of varying thickness. At the incisal border of the hypoplasia, islands of amorphous

masses are found in the ameloblastic layer. Incremental lines in the enamel matrix were observed incisally to the hypoplasia in some instances (table 1).

Accentuated incremental lines in the dentin (X in fig. 1, A, B, C and D), corresponding to the injection were found in all animals receiving 100 mgm. per kgm. or more. They are more distinct in the middle than in the apical and incisal part of the incisor; accentuation was also greater in the labial than in the lingual side.

B. Injection of boron compounds. All animals gained weight. Three animals injected with 5 per cent borax solution showed after 9 days a dry skin ulcer on the site of injection. Possibly this effect is referable to the high pH of this solution (table 2).

Accentuated incremental lines in the enamel have been found in three instances of highest concentration of boron. Only one case (rat 18) of seven investigated showed an enamel hypoplasia.

Four cases of seven showed an accentuated incremental line in the dentin. The line is most distinct at the incisal half of the labial side.

DISCUSSION. All animals injected with manganese chloride in a dosage greater than 180 mgm./kgm. showed enamel hypoplasia. After injection of boron only one animal of seven showed any such lesion and this animal received a comparatively low dosage; from this, one can safely conclude that the hypoplasia in the latter animal was probably unrelated to the injected boron. Enamel hypoplasia is even found, though rarely, in control animals. The observation that boron compounds apparently had no effect on the growth processes of the rat incisor might be significant. The association of boron and fluorine in natural waters (8) suggests that any effects of these waters cannot be attributed to the boron content. In a recent publication (9) it has been shown that boron compounds form a water-soluble complex with riboflavin, and that these preparations are suitable for injection. The findings that are reported herein indicate that the boron content of such preparations would not disturb the development of the teeth.

In most instances the subcutaneous injection of both manganese and boron solutions produces severe lesions at the site of injection leading to an ulcer of the skin. Since enamel hypoplasias were produced in the animals receiving manganese chloride but not in those receiving boron, it may be assumed that the disturbance in enamel formation was due primarily to manganese and cannot be associated with the skin lesion.

The morphologic changes of the ameloblasts in the area of hypoplasia were not constant. The ameloblasts varied from spindle-shaped to cuboid and from cuboid to high columnar. In the case of the single boron animal, the ameloblasts covering the hypoplastic matrix showed no marked morphologic changes. Variations were observed not only in sections from different animals but even in the same specimen. The different morphologic changes of the ameloblasts could not be correlated with the length of the experiments (6 to 9 days) or the dosage (table 1).

In all instances where enamel hypoplasia was found, the matrix underlying

the lesion was cuneiform in shape with the base directed incisally, and sharply bounded against normal enamel. The base of the wedge never attained the full thickness of completed enamel matrix. The hypoplastic area produced by manganese differs markedly from that produced by a single injection of strontium (10). In the strontium experiments, the hypoplasia extends over the entire zone in which the ameloblasts were producing enamel matrix at the time of injection (fig. 2, A). This zone is approximately 2 mm. long. The base of the wedge-shaped hypoplastic area, after injection of strontium, has the thickness of fully developed enamel. When, after the injection, formation of new enamel matrix begins, it is produced only apically to the hypoplastic zone. (E in fig. 2A) The strontium hypoplasia, therefore, is sharply bounded only at the apical end. In contrast to these findings, the hypoplasia produced by manganese is much shorter (about 0.5 mm.) and involves only the apical one-fourth of the zone of matrix formation (fig. 2, B). When matrix formation begins after the injection, matrix is not only laid down apically to the hypoplasia
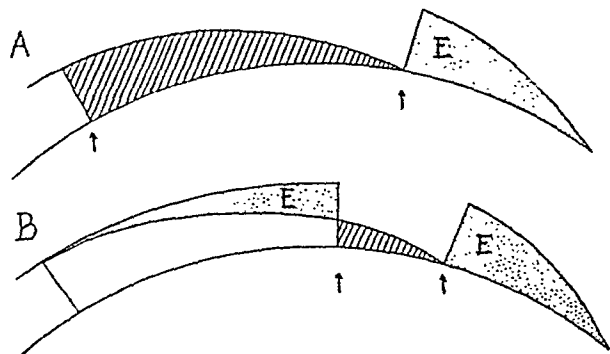

Fig. 2. After single strontium chloride injection (A) all ameloblasts forming enamel at the time of injection cease functioning and new enamel (E) is formed only apically of the hypoplasia (areas between the arrows). After a single manganese chloride injection (B) only the ameloblasts in the apical one-fourth of the formative area are affected. New enamel (E) is formed not only apically from the hypoplasia but also incisally.

but also incisally until the matrix has attained a normal thickness, (E in fig. 2, B). Therefore, the manganese hypoplasia is not only shorter than hypoplasia produced by strontium but it is demarcated sharply on both its ends by normal enamel. In the case of strontium, all ameloblasts which are actively engaged in the formation of enamal matrix are affected by the drug. On the other hand, in the animals receiving manganese, only a part of such ameloblasts were affected and these in the apical one-fourth of the area where enamel matrix formation was taking place. This varying degree of susceptibility of ameloblasts of different age to various agents may be of significance.

SUMMARY

1. Manganese chloride administered subcutaneously to white rats in dosages greater than 180 mgm./kgm. produces enamel hypoplasia.

2. The lethal dose of manganese chloride for white rats is approximately 800 mgm./kgm.

3. The subcutaneous administration of borax and/or boric acid with a total boron content of less than 200 mgm/kgm. does not cause enamel hypoplasia.

4. The injection of manganese interrupts amelogenesis in the apical one-fourth of the zone of matrix formation. This is in contrast to hypoplasia produced by strontium which extends over the whole formative zone.

5. Various agents may affect ameloblasts in different stages of their life cycle.

REFERENCES

(1) BERTRAND, G. AND M. ROSENBLATT. Am. Inst. Pasteur **35**: 815, 1921; ibid. **36**: 230, 494, 1922.

(2) McHARGUE, J. S. J. Agric. Research **24**: 781, 1923.

(3) REIMAN, C. K. AND A. S. MINOT. J. Biol. Chem. **42**: 329, 1920.

(4) BLUMBERG, H., D. H. SHELLING AND D. H. JACKSON. J. Nutrition **16**: 317, 1938.

(5) BAMANN, E. AND E. HEUMUELLER. Naturwissenschaften **28**: 535, 1940.

(6) SHINE, J. W. Plant physiol. **16**: 435, 1941.

(7) HOVE, E., C. A. ELVEHJEM AND E. B. HART. This Journal **127**: 689, 1939.

(8) SCHOFIELD AND WILCOX. Quoted after H. V. SMITH. The chemistry of fluorine as related to fluorosis in Fluorine and Dental Health. A. A. A. S. Washington, D. C. 1942.

(9) FROST, D. V. J. Biol. Chem. **145**: 693, 1942.

(10) WEINMANN, J. P. Unpublished data.

COMPARISON OF SULFOCYANATE WITH RADIOACTIVE CHLORIDE AND SODIUM IN THE MEASUREMENT OF EXTRACELLULAR FLUID[1]

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The apparent volume of distribution of intravenously injected sulfocyanate has been widely used as a measure of the volume of the extracellular fluid (1, 2, 3, 4, 5). The volume of distribution of this substance four hours after injection is fairly reproducible in the individual animal, even though the volume may fluctuate considerably after twenty-four hours (5). The proportion of the body weight corresponding to the water through which sulfocyanate is apparently distributed after four hours varies markedly, however, from animal to animal (1, 3, 4, 5). Often it clearly exceeds the usual extracellular volume of the dog, based on analyses of the water, sodium, and chloride content of whole animals (6). Its identification with the true extracellular fluid volume is, therefore, open to question.

The use of the radioactive isotopes of chloride and sodium, Cl^{38} and Na^{24}, has permitted a new approach to this problem. The chemical behavior of these radioactive isotopes is indistinguishable from that of the naturally occurring isotopes, while the radioactivity of the amounts used for tracer purposes is too small to have a demonstrable biological effect (7). Salts of Cl^{38}, of Na^{24}, and of SCN were therefore injected intravenously into dogs and their respective volumes of distribution followed during the next few hours. Variations in each volume and the relative size of the three volumes with alterations of the true extracellular fluid have been studied in this manner.

PROCEDURE. One adrenalectomized, and two normal lean, female dogs were the principal experimental subjects. Sodium sulfocyanate in 1 per cent solution was injected intravenously from a calibrated burette, followed by a solution containing radioactive Cl^{38} from a calibrated syringe. The latter solution was prepared by dissolving solid LiCl which had just been bombarded with deuterons in the cyclotron. A known amount of a solution of sodium chloride, containing Na^{24}, was injected one to twenty-four hours after the injection of Cl^{38} and SCN, and its distribution similarly determined. At one, two, and four hours after each injection samples of blood were drawn from the jugular vein, while at the same time urine was obtained by catheterization. In the earlier experiments

it was found that negligible amounts of the substances injected were excreted in the urine during the first two hours after injection. In the later experiments urine was therefore obtained only after four hours. Volumes of distribution of Cl^{38} could only be studied for two hours after injection because of the short half-life of this isotope (37 min.); those of Na^{24} and SCN were studied for four hours.

Volumes of distribution of these substances were determined in the two normal dogs both before and after twelve days of complete deprivation of food and water. In other experiments with these animals, an isotonic solution of glucose was introduced into the peritoneal cavity and permitted to remain there for four hours, at which time an equal amount of fluid was withdrawn. Since this fluid now contained sodium and chloride, the net result of this procedure was the removal of salt without water, and a consequent considerable reduction of extracellular fluid over the next three days. The volumes of distribution of the several test substances were determined before and again three days after this procedure. In the adrenalectomized dog the volumes of distribution of Cl^{38} and of SCN were obtained before and after treatment with desoxycorticosterone acetate and with cortical extract.

METHODS AND CALCULATIONS. Chloride in serum was determined by Hald and Patterson's modification of the Van Slyke method (8), in urine by the method of Volhard and Harvey (9). Sodium in serum was determined by the method of Hald (10), in urine by that of Butler and Tuthill (11). Sulfocyanate in both serum and urine was determined by the method of Crandall and Anderson (1), as modified by Elkinton and Taffel (5).

The concentration of the radioactive isotopes, Cl^{38} and Na^{24}, were determined with a Geiger-Müller counter by a method which has previously been described (12). A sample of the solution injected was diluted until it gave a count in the range of maximum accuracy of the counter; all samples of serum and urine were then diluted until they gave counts in the same general range. Volumes of distribution at any time, $E_{Cl^{38}}$ and $E_{Na^{24}}$, were calculated by the formula:

$$E = \frac{(R_i \times D_i \times V_i) - (R_u \times D_u \times V_u)}{R_s \times D_s \times \text{Donnan factor}} \div W_s$$

where R refers to the count per minute (corrected to zero time for radioactive decay), D to the dilution factor, and V to the volume. The subscript i refers to the solution injected, u to the urine, and s to the serum. W_s is the proportion of water in serum. The Donnan factor used for Cl^{38} was 1.05, that for Na^{24} was 0.95.

The difficulties involved in the calculation of the volume of distribution of sulfocyanate have been discussed in detail elsewhere (5). The theoretical formula for the apparent volume of distribution is:

$$E_{SCN} = \frac{\text{Amount given} - \text{amount excreted}}{\dfrac{\text{Rise in serum concentration}}{\text{Water content of serum}}} \times \text{Donnan factor} \times (1 - \text{fraction bound})$$

The denominator represents the rise in concentration in extracellular water, which in turn is assumed to be identical with the ultrafiltrate of serum. In the sera of the particular normal dogs used in the present study, the concentration in ultrafiltrate was determined directly and was found to be approximately $0.90 \times$ serum concentration (or $0.83 \times$ serum water concentration) (13). Therefore the following simplified formula for the apparent volume of distribution of sulfocyanate, E_{SCN}, was employed in this study:

$$E_{SCN} \frac{\text{Amount given} - \text{amount excreted}}{\text{Rise in serum concentration} \times 0.9}$$

Changes in the volume of the extracellular fluid were also independently calculated from the chemically determined balances of chloride and sodium (14):

$$E_{b_{Cl_2}} = \frac{(E_{Cl_1}38 \times Cl_1) + b_{Cl}}{Cl_2}$$

$$E_{b_{Na_2}} = \frac{(E_{Na_1}24 \times Na_1) + b_{Na}}{Na_2}$$

where:

$E_{Cl_1} 38$ and $E_{Na_1} 24$ = distribution volumes of Cl^{38} and Na^{24} respectively before the change,

b_{Cl} and b_{Na} = balances of Cl and Na.

Cl_1 and Cl_2 = initial and final concentration of Cl in extracellular water,

Na_1 and Na_2 = initial and final concentration of Na in extracellular water.

The concentration of Cl and Na in extracellular water, ECW, were calculated from the serum water concentration by the use of a Donnan factor of 0.95:

$$Cl_{ECW} = Cl_s/(W_s \times 0.95),$$
$$Na_{ECW} = (Na_s \times 0.95)/W_s,$$

where W_s = kilograms of water in a liter of serum.

By all these chemical methods, as well as by the volumes of distribution of Cl^{38}, Na^{24}, and SCN, the change in volume of the extracellular fluid was calculated as:

$$\Delta E = E_2 - E_1,$$

where E_2 and E_1 = final and initial volumes respectively.

ΔE as plotted in figure 1 is the average of the differences between the volume of distribution at one and at two hours after injection of the substances.

RESULTS. The essential data are summarized in table 1. These may be analyzed in several ways.

a. *Changes in the volumes of distribution with time.* The single determinations of the volumes of distribution of Na^{24} and SCN after one-half hour were lower than those obtained later, suggesting that equilibrium had not yet been attained.

TABLE 1

Apparent volumes of distribution of Cl^{38}, of Na^{24}, and of SCN; serum concentrations and net balances of Cl and Na

DAY OF EXPT.	CONDITION	HOUR AFTER INJECTION	WEIGHT	$E_{Cl^{38}}$	$E_{Na^{24}}$	E_{SCN}	E_{bCl}	E_{bNa}	WEIGHT	$E_{Cl^{38}}$	$E_{Na^{24}}$	E_{SCN}	E_{bCl}	E_{bNa}
			Dog 1. Normal						Dog 2. Normal					
			kgm.	liters	liters	liters	liters	liters	kgm.	liters	liters	liters	liters	liters
1	Hydrated	½	11.21			4.38			11.43			3.69		
		4	10.98			4.65			11.28			4.13		
5		½	10.84	2.76					11.40	2.58				
6		½	10.88		2.20				11.52		2.78			
		2	10.62		3.09				11.48		3.30			
		4	10.61		2.95				11.40		3.00			
19	Hydrated	1	11.04	2.86		4.18			11.38	2.70		4.12		
		2	11.01			4.19			11.34			3.97		
		4	10.94			4.22			11.28			3.99		
20		1	11.20		3.16				11.42		2.76			
		2	11.14		3.19				11.40		3.23			
		4	11.12		3.18				11.54		3.13			
27	Hydrated	1	11.50	2.94		3.89			11.52	2.86		3.75		
		2	11.70	3.27		4.20			11.42	2.83		3.91		
		4	11.50			4.25			11.44			3.83		
28		1	11.36		3.52				11.44		2.97			
		2	11.36		3.35				11.44		2.80			
40	Deprived of water and food 12 days	1	8.90	2.17		3.31			9.14	2.09		2.99		
		2, 1	8.88		2.75	3.60	2.32	2.81	9.06		2.60	3.08	2.32	2.26
		2	8.86		2.60		2.63	2.65	9.04		2.52		2.30	2.10
54	Rehydrated and fed	1	10.20	2.68		3.93	2.79		10.50	2.36		3.54	2.91	
		2	10.14	2.63		3.99	3.14		10.42	2.36		3.53	2.88	
		1	10.14		3.60				10.36		3.44			
		4, 2	10.10		3.60	3.96			10.36		3.28	3.63		
		4	10.00		3.50				10.32		3.35			
75	Hydrated	1	10.34	2.62		3.67			11.06	2.33		3.56		
		2	10.26			3.78			11.00			3.59		
		1	10.22		2.89				10.82		2.71			
		2	10.20		3.04				10.76		2.83			
78	Salt withdrawal 3 days	1	9.34	1.64		3.01	2.11	2.18	9.80	1.68		2.80	1.70	1.80
		2, 1	9.30		2.40	3.44		2.34	9.74		2.03	2.77		1.93
		2	9.32		2.55				9.68		2.16			

DAY OF EXPT.	CONDITION	HOUR AFTER INJECTION	$E_{Cl^{38}}$	E_{SCN}	DAY OF EXPT.	NET BALANCE		SERUM CONCENTRATION			NET BALANCE		SERUM CONCENTRATION		
						Cl	Na	H₂O	Cl	Na	Cl	Na	H₂O	Cl	Na
	Dog 3. Adrenalectomized					Dog 1. Normal					Dog 2. Normal				
			liters	liters		m. Eq.	m. Eq.	gms. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq.	m. Eq.	gms. per liter	m. Eq. per liter	m. Eq. per liter
1	No treatment	1	2.04*	2.84*	28			938	108.1	142.7			944	113.3	144.9
11	DOCA + cortin	1	2.62†	3.29†	40	—62	—80	932	112.8	149.6	—55	—74	934	117.0	156.2
24	DOCA + cortin	1	2.65†	3.47†	54	+33		938	104.5	142.2	+39		944	106.4	137.5
33	DOCA	2½	2.76†	3.64†	75			939	108.9	140.0			942	111.2	137.5
					78	—86	—103	929	98.5	137.3	—124	—133	920	85.4	120.5

* Serum water content assumed to be 910 grams per liter.
† Serum water content assumed to be 930 grams per liter.

The volumes of distribution of these two substances sometimes changed between the first and second and again between the second and the fourth hours after injection. These changes were never large, however, and were not consistent in direction. It therefore seems more reasonable to ascribe such variations to technical difficulties or to true changes in the volumes through which the substances were distributed, than to lack of equilibrium between plasma and interstitial fluid after one hour. Of the four determinations of the volume of distribution of Cl^{38} at one and again at two hours after injection, three were identical and one was slightly greater after two hours, suggesting that for this substance, too, equilibrium is usually complete after one hour.

b. *Relative magnitudes of the volumes of distribution of* Cl^{38}, Na^{24} *and SCN.* Under all conditions of hydration the volume of distribution of Cl^{38} was uni-
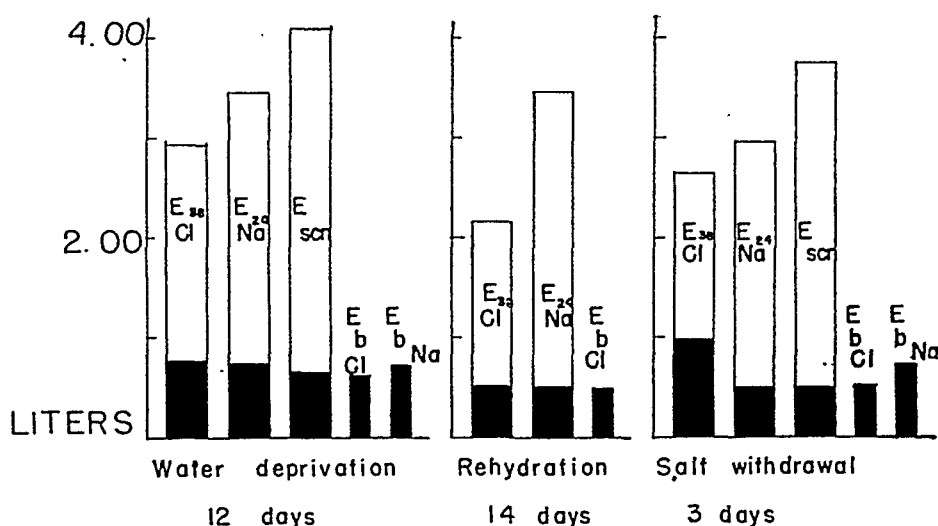

Fig. 1. Comparison of measurements of changes in extracellular fluid volume in dog 1. Blank columns represent the initial absolute magnitude of the volumes of distribution of Cl^{38}, Na^{24}, and SCN, before change has occurred. Black columns represent the changes, $\Delta E_{Cl^{38}}$, $\Delta E_{Na^{24}}$, ΔE_{SCN}, ΔE_{bCl} and ΔE_{bNa}. In the first and third experiments the changes were negative, in the second, positive. The results with dog 2 are similar.

formly the smallest, and that of SCN the largest of the three substances. In terms of per cent of body weight, the average volumes of distribution of the three substances in dogs 1 and 2 when normally hydrated were: 24.7, 27.6, and 35.6 for Cl^{38}, Na^{24}, and SCN respectively.

A statistical analysis of the difference between the volumes of distribution of the three substances was carried out. $E_{Cl^{38}}$ was taken as a standard for reference. In the three dogs, hydrated and dehydrated, the mean of 34 individual ratios of $E_{Na^{24}}$ to $E_{Cl^{38}}$ was 1.25, with a standard deviation of ± 0.16. Similarly, of 38 individual ratios of E_{SCN} to $E_{Cl^{38}}$ the mean ratio was 1.52 with a standard deviation of ± 0.16. The difference between E_{SCN} and $E_{Cl^{38}}$ is therefore somewhat more significant statistically than that between $E_{Na^{24}}$ and $E_{Cl^{38}}$.

c. *Effect of changes in hydration on the volumes of distribution of* Cl^{38}, Na^{24}

and SCN. Changes in the distribution volumes of Cl^{38}, of Na^{24} and of SCN were compared under conditions known to produce changes in the volume of extracellular fluid. Changes were induced in each of the two normal animals, once by water deprivation, and once by salt withdrawal by intraperitoneal glucose solution. The changes were also measured by chloride and sodium balances over the three periods. Despite large differences in the initial distribution volumes of the three substances, the changes as measured in two to five ways were always in the same direction and were of approximately the same magnitude (fig. 1). Similarly the changes in the volumes of distribution of Cl^{38} and SCN, produced by treatment in the adrenalectomized dog, dog 3, over 33 days were + 0.72 liter for $\Delta E_{Cl^{38}}$ and + 0.80 liter for ΔE_{SCN}.

Although the changes as measured by the different methods agree for the most part, there are some discrepancies which appear to bear no relation to the initial absolute magnitude of the distribution volumes or to any one of the substances used. That is, there are occasional single differences from the average value of as much as −48 and +74 per cent. The data on changes are too few to be submitted to a satisfactory statistical analysis.

DISCUSSION. The results of these experiments are perhaps best understood if it is recalled that the extracellular fluid volume is the summation for the whole organism of a large number of particular fluid phases in many different kinds of tissue. The distributions of chloride, sodium, and sulfocyanate undoubtedly vary from one tissue to another. Sulfocyanate is definitely known to enter the cells of certain tissues, such as erythrocytes and gastric mucosal cells (1, 2), and indirect evidence has been presented that it probably enters the cellular phase of other tissues as well (5). Sodium is present in the dog erythrocyte (15) and in the cells of striated muscle (6). Cohn and Cohn (16) have shown that Na^{24} rapidly penetrates dog erythrocytes. Manery and Bale (17) in an extensive study of the penetration of Na^{24} into the different phases of many types of tissues conclude that although its entrance into some sodium-containing cells is slow, in others, especially secretory cells, the entrance is rapid. Greater cellular penetration in certain tissues must explain the larger volumes of distribution of sodium and of sulfocyanate.

Other things being equal, in experiments of this type the substance giving the smallest volume of distribution is logically the best measure of the extracellular fluid. Chloride, on this basis, should be the substance of choice. But chloride itself is known to enter erythrocytes, and hence must give a measurement which is somewhat too large. Chloride is also known to be present in the cells of connective tissues, pyloric mucosa, and testes (18). However, as Manery and Haege (19) have shown that in the case of rat testes and rabbit pyloric mucosa, Cl^{38} in the first few hours penetrates only the sodium or extracellular phase, intracellular distribution of chloride in these tissues probably does not occur over the two hour interval of these experiments. At present we can only state that any substance, such as sulfocyanate, whose volume of distribution exceeds that of radioactive chloride, is unsatisfactory in the absolute measurement of extracellular fluid. This does not preclude its use as a relative measure of

changes in the extracellular fluid volume, and our evidence on the whole supports the validity of the use of sulfocyanate for this purpose.

However, these experiments indicate that serial determinations of the volume of distribution of some single test substance may give misleading values for changes in the extracellular fluid volume. If such changes are to be studied, it is desirable to check changes in the distribution volume of sulfocyanate or of any other test substances·by chloride and sodium balances.

The conclusion should not be drawn that the ratios of the absolute magnitudes of the volumes of distribution of Na^{24} and SCN to Cl^{38} are 1.25 and 1.52 respectively in all or any dogs. These data were obtained from only three dogs. A review of all the determinations of the distribution of sulfocyanate in normal hydrated dogs in this laboratory since the present SCN method has been in use shows that, in terms of body weight, the average value for E_{SCN} was 34.6 per cent with a maximum of 42.3 per cent and a minimum of 26.7 per cent. These figures are from 29 determinations, made one to four hours after injection in 12 dogs, and include those reported in this paper and in a previous paper (5). This wide range of values agrees well with those found by other workers; values of 27 to 36 per cent, 23.0 to 42.5 per cent, and 21.2 to 37.0 per cent of the body weight having been reported by Crandall and Anderson (1), Gregersen and Stewart (3), and Mellors et al. (4), respectively. In view of this wide variation the volume of distribution of SCN and its relation to that of Cl must vary considerably in different dogs.

Although fairly constant volumes of distribution of SCN have been obtained at one to four hours after repeated injections over the course of a few weeks, there is some evidence that over a longer period of time the cellular phases into which SCN penetrates rapidly during the first four hours may become relatively saturated after repeated injections. Thus in table 1 E_{SCN} fell gradually over an eleven week period from 43.3 per cent of the body weight to 35.5 per cent in dog 1 and from 36.6 per cent to 32.2 per cent in dog 2. Six months after the first of these determinations E_{SCN} was 30.4 per cent and 26.7 per cent of the body weight in these two dogs respectively.

Kaltreider, Meneely, Allen and Bale (20) have studied the relation of the volumes of distribution of radioactive sodium and of sulfocyanate in man. These workers found that both volumes, after a sharp rise during the first three hours, reach a fairly constant level during the next nine hours. The magnitudes of the two volumes were very similar and approximated 25 per cent of the body weight. The difference between their results in man and our results in the dog may be due to the difference in species.

SUMMARY AND CONCLUSIONS

In three dogs under varying conditions of hydration the absolute magnitude of the apparent volume of distribution of sulfocyanate always exceeded that of radioactive sodium, which in turn exceeded that of radioactive chloride.

In the hydrated normal dogs Cl^{38}, Na^{24}, and SCN were distributed through

approximately 25 per cent, 28 per cent and 36 per cent of the body weight respectively.

In spite of these differences in the absolute magnitude, changes in the volume of the extracellular fluid as measured by each of the three substances and by chloride and sodium balances were usually of about the same magnitude; occasionally unexplained discrepancies appeared.

The volume of distribution of sulfocyanate is therefore not a satisfactory absolute measure of the volume of the extracellular fluid. Under most conditions, however, it may be a useful relative measure, i.e.; changes in its volume of distribution may reflect changes in extracellular fluid.

REFERENCES

(1) CRANDALL, L. A., JR. AND M. X. ANDERSON. Am. J. Digest. Dis. and Nutrition 1: 126, 1934.

(2) LAVIETES, P. H., J. BOURDILLON AND K. A. KLINGHOFFER. J. Clin. Investigation 15: 261, 1936.

(3) GREGERSEN, M. I. AND J. D. STEWART. This Journal 125: 142, 1939.

(4) MELLORS, R. C., E. MUNTWYLER, F. R. MAUTZ AND W. E. ABBOTT. J. Biol. Chem. 144: 785, 1942.

(5) ELKINTON, J. R. AND M. TAFFEL. This Journal 138: 126, 1942.

(6) HARRISON, H. E., D. C. DARROW AND H. YANNET. J. Biol. Chem. 113: 515, 1936.

(7) HAMILTON, J. G. AND G. A. ALLES. This Journal 125: 410, 1939.

(8) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. II, Methods, p. 838, Williams & Wilkins, Baltimore, 1932.

(9) HARVEY, S. C. Arch. Int. Med. 6: 12, 1910.

(10) HALD, P. M. J. Biol. Chem. 103: 471, 1933.

(11) BUTLER, A. M. AND E. TUTHILL. J. Biol. Chem. 93: 171, 1931.

(12) EISENMAN, A. J., L. OTT, P. K. SMITH AND A. W. WINKLER. J. Biol. Chem. 135: 165, 1940.

(13) LAVIETES, P. H., J. R. ELKINTON AND T. S. DANOWSKI. Unpublished data.

(14) LAVIETES, P. H., L. M. D'ESOPO AND H. E. HARRISON. J. Clin. Investigation 14: 251, 1935.

(15) KERR, S. E. J. Biol. Chem. 117: 227, 1937.

(16) COHN, W. E. AND E. T. COHN. Proc. Soc. Exper. Biol. and Med. 41: 445, 1939.

(17) MANERY, J. F. AND W. F. BALE. This Journal 132: 215, 1941.

(18) MANERY, J. F. AND A. B. HASTINGS. J. Biol. Chem. 127: 657, 1939.

(19) MANERY, J. F. AND L. F. HAEGE. This Journal 134: 83, 1941.

(20) KALTREIDER, N. L., G. R. MENEELY, J. R. ALLEN AND W. F. BALE. J. Exper. Med. 74: 659, 1941.

THE CENTRIFUGAL COURSE OF WALLERIAN DEGENERATION

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In a previous report (Rosenblueth and Dempsey, 1939) Parker's (1933) conclusion was confirmed that Wallerian degeneration is faster at more central than at more peripheral regions of a severed axon. The question arises whether the faster rate of degeneration near the section of the nerve is determined by the section itself, or whether it depends on a gradient intrinsic to the axons. This study attempts to answer that question.

METHOD. In cats under ether or nembutal anesthesia the peroneal nerves were cut at one or more of the following regions: the hip, the middle of the thigh, and the knee, before the nerve begins to distribute its branches. These sections were made with aseptic precautions. Care was taken not to cut the arteries which accompany the nerves, in order to preserve the blood supply.

Two to four days later, the cats were anesthetized with dial (Ciba, 0.7 cc. per kgm., intraperitoneally). The degenerating peroneals were dissected from hip to knee. Depending on whether or not the section at the middle of the thigh had been made, one (10 to 12 cm.) or two (5 to 6 cm.) stretches were thus available for study from each side of the animal. After crushing the ends of each stretch they were placed on electrodes in a moist chamber.

The nerves were stimulated maximally by brief condenser discharges with a frequency of 10 to 30 per sec. This frequency was regulated by a thyratron which tripped the stimuli and also the sweep of the cathode-ray oscillograph.

The electric responses were led monophasically or diphasically to the amplifier. Capacity-coupled amplification was employed, with a time constant of 0.2 sec. The responses were photographed from the standing waves in the face of the oscillograph. Each picture corresponded to from 3 to 6 superimposed responses. The description of the position of the stimulating and recording electrodes is given with the corresponding experimental results.

The areas underlying the monophasic responses were measured as follows. The records were projected through a photographic enlarger and were drawn on light cardboard (filing cards). They were then cut out and were weighed to ± 5 mgm. The average weight was about 250 mgm. Since the sweep was not strictly linear (see figs. 1*f* and 3 D) a correction of these weights was made by dividing their value by the speed of the sweep at the region where the peak of the spike was located. The latter figure was closely proportional to the area of the responses.

The observations were made mainly on the A fibers of the nerves and only incidentally on B axons.

RESULTS. A. *The degeneration gradient in nerves with a single cut at the hip.* In the study of Rosenblueth and Dempsey (1939) the existence of this gradient was concluded from a comparison of the amplitude of the spike potentials of the degenerating trunks with those of normal controls when the nerves were stimulated at different regions from center to periphery. In the present observations more accurate methods were used, as follows.

If in normal nerves the recording electrodes are kept in a fixed position on intact and crushed regions, respectively, the amplitude of the spikes decreases gradually as the stimulating cathode is moved away from the recording region (fig. 1A). As is well known, this decline of amplitude is due to increasing
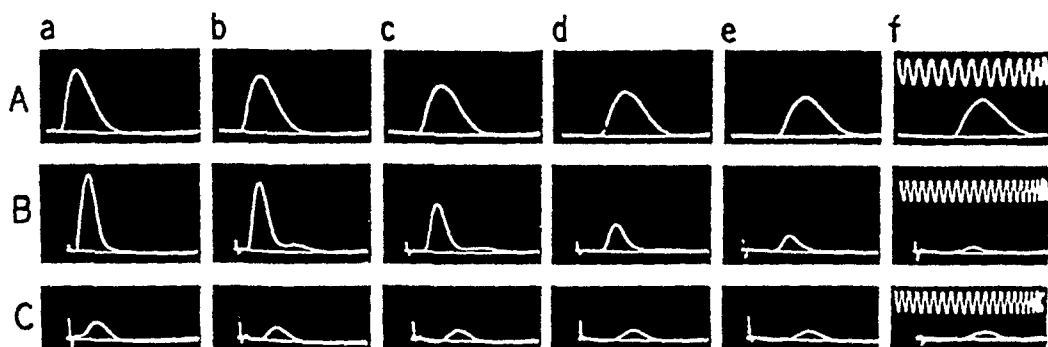

Fig. 1. Changes of amplitude of maximal monophasic spike potentials with variable conduction distance. The time of stimulation is indicated by the stimulus artifacts in B and C. In A this artifact was too small and does not appear. Its position was fixed and occurred shortly after the beginning of the sweeps. The speed of the sweeps is calibrated for A, B and C, respectively by the sinusoidal waves (5,000 cycles) at the top of *f*. In these and other records the horizontal line corresponds to the level of the oscillograph for the resting nerve.

A. Normal peroneal. Record from the peripheral end (interelectrodal distance 1.5 cm.). Distances between the stimulating cathode and the proximal recording electrode: *a* to *f*, from 2 to 7 cm. by 1 cm. steps.

B. Degenerating peroneal cut both centrally (hip) and peripherally (knee) 4 days previously. Records as in A.

C. The same degenerating nerve as in B, but records from the central end. Amplification increased. Conduction distances from 2.5 to 7.5 cm.

temporal dispersion. The areas of the recorded response, however, decrease only slightly with increasing conduction distance (fig. 2A).

Series of observations were made with variable stimulated points and with fixed recording electrodes at both the central and the peripheral ends of the degenerating nerves. Typical results were similar to those illustrated in figures 1 and 2. When the record was peripheral both the amplitude and the area of the spike potentials declined markedly with increasing conduction distance—i.e., with stimuli applied progressively closer to the central end. When the record was central the amplitude decreased only slightly and the areas remained practically constant as the conduction distance was increased.

The gradient of the areas with peripheral recording was steeper the more advanced the degeneration. Thus, with stimuli moved toward the central end, only a slight decrease of area was seen in nerves cut 2 days previously, while

the decrease was very pronounced in nerves studied 4 days after section. The sigmoid shape of the middle curve in figure 2A is typical. The position of the steeply ascending portion varied with the degree of degeneration. Thus, in 3-day nerves that portion was close to the central regions while in 4-day nerves it was located in more peripheral regions.

The gradient of responses supports the inference of a gradient of degeneration. With a peripheral record the responses decrease when the stimuli are moved away from the record because less and less fibers are stimulated—i.e., because
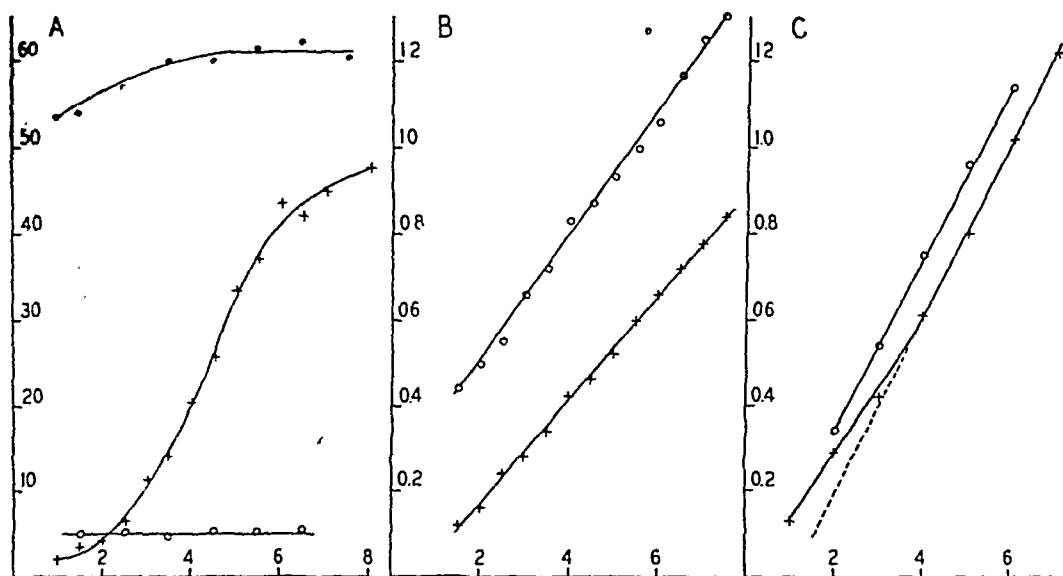

Fig. 2. A. Areas of maximal monophasic spike potentials with variable stimulated region. Ordinates: areas in arbitrary units. Abscissae: distance (in centimeters) between the stimulating cathode and the central cut of the nerves.

Upper curve (dots). Normal peroneal. Record from peripheral end.

Middle curve (crosses). Peroneal, 4 days after 2 cuts had been made, at the hip and knee, respectively. Records from the peripheral end.

Lower curve (circles). Same nerve as in B. Records from the central end.

B. Uniformity of the conduction velocity along a normal peroneal. Ordinates: latencies in milliseconds. Abscissae: conduction distances in centimeters. Lower curve: beginning of responses. Upper curve: peak of the spike potential of the A fibers.

C. Conduction velocity of fibers along a degenerating peroneal (4 days). Ordinates and abscissae as in B. Lower curve: beginning of responses with peripheral recording. Upper curve: same, but central recording.

more and more fibers have degenerated to a stage where they do not conduct. With a central record the responses do not vary much because many of the few recording fibers functional in that region will be activated throughout the nerve. Impulses set up in the functional peripheral part of other fibers will not reach the central region. The S shape of the middle curve in figure 2A indicates that there is a relatively short stretch of nerve separating the highly degenerated, nonfunctional central region, from the slightly degenerated, functional peripheral part.

An alternative method of testing the nerves was to move from center to

periphery, or vice versa, all 4 electrodes, stimulating and recording, keeping the respective distance constant—e.g., 1 cm. interelectrodal recording and stimulating distance, and 1.5 cm. conduction distance.   Except for the positions in which one of the recording electrodes was at a crushed end, the records were diphasic.

When this procedure was used in normal nerves the responses were about 30 per cent smaller from more peripheral than from more central recording regions. This difference suggests that the amplitude of the recorded spike potentials is smaller in the distal than in the central portions of the nerves.   Standard monophasic records supported that inference.   The source of this asymmetry will be discussed later.

Similar tests made on degenerating nerves led to opposite results—i.e., the responses were larger at the more peripheral than at the more central regions. These results again support the inference that at a given time after central section, more fibers are degenerated towards the centers than towards the periphery.
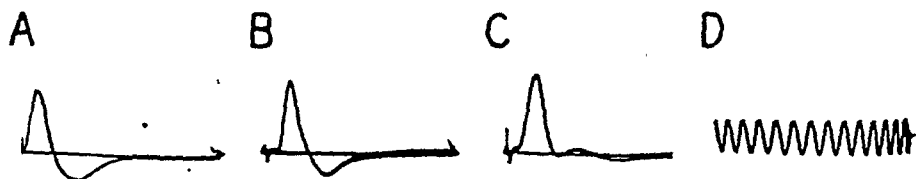

Fig. 3. Changes of diphasic responses in degenerating peroneals.   The recording electrodes were 1 cm. apart, and placed at least 2 cm. away from the cut ends.   The conduction distance was 2 cm.

A. Normal nerve.   The record begins at the end of the stimulus artifact.

B. Degenerating nerve (4 days).   Record from a relatively peripheral part.   Stimulus applied centrally.   The stimulus artifact precedes the response.

C. Degenerating nerve (4 days).   Record from a relatively central part.   Stimulus applied peripherally.

D. Five thousand cycles.

A further difference between normal and degenerating nerves was the following.   As seen in figure 3A the 2 phases of the diphasic normal electrogram are prominent.   Indeed, the areas of these 2 phases are approximately equal.   This equality obtains whether the stimuli are applied at a central or at a peripheral region, with respect to the recording electrodes.   In degenerating nerves, on the other hand, while the diphasic electrograms obtained from central stimulation resembled the normal responses (fig. 3B) those recorded with peripheral stimulation were asymmetric.   The 1st phase was greater than the 2nd one.   The records tended to become monophasic (fig. 3C).

A final difference between normal and degenerating nerves appeared when conduction-velocity measurements were carried out.   In normal nerves the conduction velocity of the fastest fibers was uniform throughout the trunks tested (lower line in fig. 2B).   In addition the latency of the peaks of the spike potentials varied uniformly with increasing conduction distance (upper line, fig. 2B), indicating that the rate of conduction for the average A fibers was uniform.   The

velocity of propagation was the same in measurements made with peripheral and with central recording, respectively.

While the graphs correlating latency of response with conduction distance were straight lines when the record was from the central end of the degenerating nerves, they could deviate significantly from the straight line when the record was from the peripheral end. This was true not only of the latency of the beginning of the responses (fig. 3C), but also of that of the peak of the A spike potential. The deviation was more striking when the degeneration was more advanced—i.e., it was seldom found before 3 days, but was the rule after 4 days of degeneration.

The conduction velocities measured in degenerating nerves were sometimes faster than normal when degeneration was not very advanced (up to 105 m/sec. in some of the 3 day nerves; cf. table 1). This confirms similar observations of Rosenblueth and Dempsey (1939). With more advanced degeneration the

TABLE 1

Conduction velocities (m/sec.)

The rows indicate the propagation velocity of the beginning of the responses (*fastest* fibers) and of the *peak* of the A spike potential (average A fibers). In the columns the symbols mean as follows: 3 and 4 days, time of degeneration; C and P, central or peripheral recording; c and p, stimulation of a relatively central or peripheral region. Each figure is the average of from 5 to 11 measurements in different nerves. There was little scatter except for the columns corresponding to 3 days of degeneration.

	NORMAL NERVES	3 DAYS·		4 DAYS		
	C and P	C	P	C	Pc	Pp
Fastest....................	82	75	83	55	49	73
Peak......................	62	63	65	45	44	72

rate of propagation was slower than normal throughout the nerve, but it was slower in the central than in the peripheral regions (table 1).

The irregular lower line of figure 2C is readily explained by the interpretation that the larger fibers, which conduct more rapidly, are totally degenerated in the central portions, while some of them are still functional in the peripheral regions. The two slopes in the line thus probably denote two sets of fibers, not a change of velocity in the same elements.

B. *The gradient in nerves with central and peripheral cuts, at hip and knee.* As illustrated in figures 1, 2 and 3, all the tests described in the previous section yielded results in these nerves similar to those obtained with only one cut at the hip. In some animals the double section was made on one side while only a central cut was made on the other. A comparison of the two nerves showed only minor quantitative differences. It may be inferred, therefore, that the peripheral cut at the knee does not modify the centrifugal course of Wallerian degeneration.

C. *The nerves with a central and a middle cut, at hip and middle thigh.* The purpose of these observations was to see whether a single gradient would obtain

for the nerve as a whole, from hip to knee, or whether each cut would set-up two similar gradients, one for the upper and the other for the lower segment of the nerve. In other words, the question was whether or not the degree of degeneration would be the same at the central end of the two segments divided by the middle cut—i.e., immediately below the cut at the hip and below that in the middle.

The results indicated clearly that a single gradient was present for the nerve as a whole. Thus, after 2 or 3 days of degeneration the upper segment usually showed a marked centrifugal gradient while the lower one did not show it—i.e., the lower segment was practically undegenerated throughout its extent, while the upper one showed extensive degeneration toward the central end.

After more prolonged degeneration (3 to 4 days), the A fibers of the upper segment as a rule were almost totally degenerated while a sizable fraction of them were functional in the lower portion.

The amplitude of the monophasic responses recorded with a standard procedure was invariably greater at the lower central region (below the middle cut) than at the upper one (below the cut at the hip). The responses at about 1 cm. below the middle cut were only slightly smaller than those at about 1 cm. above this section.

It may be inferred, therefore, that an additional middle cut does not modify the course of degeneration of the nerve as a whole consequent to a higher section at the hip.

DISCUSSION. Some considerations of the structure and properties of normal peroneal nerves, in the regions studied, are necessary for the evaluation of the results on the degenerating trunks. Specifically, the question arises whether this part of the peroneal may be considered a homogeneous, uniform trunk, or whether there is a systematic anatomical change in the A axons from center to periphery. This question is suggested by the observation that the amplitude of responses is smaller at more peripheral than at more central parts of normal nerves (p. 250). A change in the average diameter of the axons along the nerve would result in a change of the amplitude of the spike potential (see Gasser and Grundfest, 1939).

The observations of Eccles and Sherrington (1930) oppose the view that there is a significant change of the average diameter size of the axons in the regions of the nerves tested. They found only a slight degree of branching, and hence a slight decrease of diameter, of motor axons measured between 9 and 62 mm. away from the gastrocnemius muscle. This branching was more marked in the regions close to the muscle than in more central parts of the nerve. The changes of diameter of the axons in the nerves studied here would therefore be practically negligible.

In support of the inference that the trunks studied are uniform are the measurements of conduction velocity illustrated in figure 2B. The latencies of the peaks of the spike potentials may be taken as an accurate basis for measurements of average conduction velocity. If the diameter of the axons decreased at the periphery their conduction velocity would become slower, as is well known. The

plot of the latencies against the conduction distance would then yield a graph which would deviate from a straight line. The straight lines in figure 2B indicate, therefore, that the conduction velocities of both the fastest and the average A fibers in the nerves remained uniform throughout the trunk. They indicate, therefore, that there is no significant progressive decrease of diameter of axons from center to periphery in this part of the nerves.

It may be concluded from this argument that the smaller amplitude of responses recorded peripherally, as compared to those recorded centrally, is not due to a change in diameter of the fibers. An alternative, more likely, explanation is that as the nerve begins to organize into its several peripheral branches there is an increase in the relative amount of fascia surrounding the axons. This fascia acts as a shunt which decreases the recorded potentials. A further corollary of the foregoing discussion is that the gradient of degeneration revealed by the data is not a consequence of a non-uniform structure of the axons.

Since the blood supply of the nerves was carefully preserved at the time of the sections, it may be concluded that the centrifugal gradient of degeneration is not determined by changes in the irrigation of the tissue.

That the gradient is not determined by the section of the nerves is indicated by the fact that it was not modified when several cuts were made, instead of only a central section (figs. 1 and 2, p. 251). The lack of influence of additional sections excludes the diffusion at the cuts of some substance into or out of the axons as a possible factor for the asymmetry in the progress of degeneration. The results lead to the inference that the centrifugal course of degeneration is due to a polarization or gradient which is intrinsic to the axons. The nature of this gradient is obscure at present. An obvious working hypothesis is that it is metabolic, so that more central regions of axons burn up more rapidly than more peripheral parts. Other hypotheses are possible, however, and only future study will clear the problem.

It may be pointed out that the demonstration of an asymmetry in axons, and even the knowledge of the source of this asymmetry, does not and will not clarify the reason for Wallerian degeneration. The factor or factors responsible for the degeneration of an axon separated from its cell-body and nucleus may be entirely independent of the conditions which determine the time course of that degeneration.

The relative monophasicity of responses recorded from two uncrushed points in the degenerating nerves when the stimuli are applied peripherally (fig. 3C) is attributed to the presence of axons which are functional at the region in contact with the recording electrode proximal to the stimulated part, but which are not functional at the region of the distal recording lead, because of the more advanced central degeneration. As a corollary of this interpretation it may be inferred that non-conducting parts of a degenerating axon behave like injured parts of normal axons with respect to the recording of the action potential from other normal or less degenerated regions.

The present observations confirm previous reports that larger fibers degenerate more rapidly than smaller axons (see for references Spielmeyer, 1929). Thus,

the abnormal prominence of the B spike potentials in nerves with an advanced degeneration, as compared with normal trunks (cf. fig. 1 A and B) indicates more degeneration of A than of B axons. Similarly, as already mentioned (p. 249), the broken lower line of figure 2C is best explained by the assumption that some of the large, fast fibers are entirely degenerated in the central region of the nerves at a time when smaller, slower axons are still functional.

SUMMARY

The peroneal nerve of the cat is shown to have A axons of uniform diameter between the hip and the knee (p. 253). Wallerian degeneration of that nerve follows a centrifugal course—i.e., central portions degenerate more rapidly than peripheral regions (pp. 249, 250). This gradient of degeneration is uninfluenced by cuts additional to the central section at the hip (figs. 1 to 3; p. 252). The gradient, therefore, is not determined by the cuts, thus excluding the possibility of diffusion of some substance out of or into the axons as a factor. The gradient does not depend on the blood supply to the nerve (p. 253). It reveals a longitudinal asymmetry intrinsic to otherwise uniform axons.

It is confirmed that larger fibers degenerate more rapidly than smaller axons (p. 254).

Monophasic action potentials may be recorded from a functional to a degenerated region of an axon (p. 253, fig. 3).

REFERENCES

ECCLES, J. C. AND C. S. SHERRINGTON. Proc. Roy. Soc., B. **106**: 326, 1930.
GASSER, H. S. AND H. GRUNDFEST. This Journal **127**: 393, 1939.
PARKER, G. H. Ibid. **106**: 398, 1933.
ROSENBLUETH, A. AND E. W. DEMPSEY. Ibid. **128**: 19, 1939.
SPIELMEYER, W. Handbuch der normalen und pathologischen Physiologie. Vol. 9, Berlin, 1929.

AN ANALYSIS OF THE THIOCYANATE METHOD FOR DETERMINING THE DISTRIBUTION OF THE BODY FLUIDS

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Since the introduction of thiocyanate by Crandall and Anderson (4) as a substance which is distributed mainly in the extracellular fluids, the method has been used on several occasions for measuring changes in extracellular fluids (11, 12, 14, 15). Attempts at standardization of the method have been carried out by a number of investigators (8, 9, 13). As a result of these investigations the thiocyanate method is appreciated as a valuable adjunct in studying the body fluids under experimental conditions, and the method is generally accepted as valid as far as relative changes are concerned. In some recent studies (1, 2, 10) we have attempted to use the method and have ecountered some difficulties in repeated determinations of available fluid (for solution of thiocyanate). The present study therefore attempts to evaluate the method for approximation of extracellular fluid volume changes and to standardize the method for repeated determinations.

METHODS. Dogs were used as the experimental animal. Anesthesia was usually not employed, but in a few instances pentobarbital sodium intravenously was used. Thiocyanate was administered as 2.5 or 5 per cent solution of the potassium salt. The solution was measured into a syringe by means of a volumetric pipette attached to the syringe tip by a short segment of small-bore rubber tubing. The solution was given by way of the jugular vein and the syringe was rinsed twice with blood to insure complete emptying. Blood samples were taken from the jugular vein at 10 to 15 minute intervals during the first hour, at 20 to 30 minute intervals in the second hour and at variously longer intervals thereafter. Thiocyanate was determined according to the method of Lavietes, Bourdillon and Klinghoffer (9), except that adoption of the method to the Lumetron photo-electric colorimeter was made. We have found the color produced by ferric nitrate reagent to fade within a short period of time, and for this reason the color is produced in each sample separately and read in a uniform time, that is, within 30 seconds after the addition of ferric nitrate. The small amount of thiocyanate present in normal control plasma makes it necessary to make a correction, especially when reinjection of thiocyanate for repeated determination is carried out. To avoid the error, we carry out the procedure on control plasma and use this for the transmission standard instead of distilled water. Plasma thiocyanate determinations were carried out in duplicate. These usually checked within 0.1 mgm. per 100 cc. Urine thiocyanate determinations were carried out by the same procedure as for plasma with the exception that the transmission standard was urine-trichloracetic acid filtrate diluted with an equal volume of distilled water. Sodium determinations were made in duplicate by the method of Butler and Tuthill (3).

Diffusion time. Crandall and Anderson found the diffusion time to be around one hour in dogs. Stewart and Rourke (13) found the plasma level to stabilize in about 30 minutes in man. In 22 dogs in the present study the diffusion time was found to average between 47 to 67 minutes (the average times of the next to last, and last determinations, after which subsequent readings were stable). The maximum time in the series was 67 to 89 minutes. No correlation between diffusion time and size of dog or thiocyanate level was apparent.

Disappearance of thiocyanate. Crandall and Anderson report an average rate of disappearance of 0.08 mgm. per 100 cc. per hour. In 23 dogs used in this study the average rate of disappearance was found to be 0.15 mgm. per 100 cc. per hour as determined over a period of 24 hours. The difference between this figure and that obtained by Crandall and Anderson, if significant at all, may be attributed to different average plasma thiocyanate levels in the two studies. This rate of disappearance was found to vary considerably in different animals, the range being 0.04 to 0.41 mgm. per 100 cc. per hour. To some extent this variation depended upon the concentration of thiocyanate in the extracellular

TABLE 1

Comparison of total disappearance of thiocyanate from extracellular fluid with total urine excretion

EXPERIMENT	TIME	BLOOD LEVEL	DISAPPEARANCE RATE	TOTAL SCN DISAPPEARANCE IN EACH EXPT.	TOTAL AMOUNT SCN EXCRETED IN URINE
	hours	*mgm./100cc.*	*mgm./100 cc./hr.*	*mgm.*	*mgm.*
1	24	12.4 –8.8	0.17	144	21
2	6	7.25–5.7	0.29	36	7.3
3	25	11.3 –7.6	0.16	153	18.5
4	24	8.15–6.95	0.07	38	29
5	17½	10.6 –7.15	0.20	160	16

fluid so that an average per cent disappearance is somewhat more accurate in estimating the rate of disappearance. This percentage factor, in this series of 23 dogs, was found to be 2.3 per cent per hour.

Disposition of thiocyanate. No attempt was made in this study to establish the exact location of the thiocyanate ion as it disappeared from the extracellular fluid. However, some suggestion as to the disposition of the ion may be derived from observations on the following experiments. In 5 dogs quantitative determination of urine thiocyanate over a period of about 24 hours was compared with the amount which disappeared from the original available fluid, which latter was assumed to remain constant in volume. As shown in table 1, the results of this study indicate that the amount disappearing from this original available fluid far exceeds that quantity which is eliminated in the urine during the same period. According to these findings, the repeated determination of available fluid by subtracting that thiocyanate excreted in the urine from the total amount originally injected as was done by Elkington and Taffel (6) would give erroneous results. This may account for the unreliability of the thiocyanate method for

measuring a given portion of the body fluid over an extended period, as found by these authors, since false high values would be obtained by this method of correction. These data would seem to indicate, in accord with the findings of Elkington and Taffel, that thiocyanate rapidly diffuses into one portion of the body fluids and slowly enters another phase of the body fluids, probably the cells.

Relation of urine excretion of thiocyanate to plasma level. A progressive increase in thiocyanate concentration in the plasma was produced in 4 dogs by continuous intravenous administration of thiocyanate. Urine collections were taken at intervals and blood samples were simultaneously obtained. A distinct correlation of urine and plasma thiocyanate concentration was detected by this means although the urine level was always definitely lower than the level in the plasma. Thus, with increased plasma levels, one may expect a more rapid urine excretion. This, at least, partly explains the higher rates of disappearance found in the animals with higher plasma levels in the first part of this study.

Optimum thiocyanate dosage. The toxic and lethal levels of thiocyanate in the plasma vary with different animals. Rapid injection of doses of 40 to 75 mgm. per kilo may cause death. On the other hand levels of 30 mgm. per 100 cc. have frequently been attained without obvious untoward symptoms. In order to avoid toxic or lethal effects the dosage should not exceed 30 mgm. per kilo. of body weight. The optimum value in the plasma from the technical standpoint is around 8 mgm. per 100 cc. which is approximately attained by the administration of 25 mgm. per kilo of body weight in the dog.

The use of the thiocyanate test in detecting changes in the extracellular fluid. In order to test the accuracy of reinjection of thiocyanate, nine normal dogs were used. The original available fluid was determined by a single injection. In this determination precaution was taken to insure complete diffusion and correction was made for disappearance either by actually determining the disappearance rate in individual dogs or by using the factor 2.3 per cent disappearance per hour as discussed above. After completion of the first determination a second determination of available fluid was made by reinjection of thiocyanate. By taking the difference between the levels attained after reinjection and that existing before reinjection, a second calculation of available fluid was made. The results of single injection and reinjection in normal dogs in which no change in extracellular water was produced are compared in table 2. It is noticeable in the results that there is definite variation, which we consider to represent the culminative vicissitudes of the test. The errors are on both the positive and negative side, that is, neither method is consistently higher or lower. The greatest per cent of error from the first determination is 5.8 per cent and average error is plus or minus 3.6 per cent. In table 3 are presented the data on the use of the thiocyanate method in detecting changes in extracellular fluid volume by causing an increase of extracellular volume in two dogs and a decrease in three dogs. The increase in extracellular fluid volume was brought about by ligation of both ureters and injecting 1000 cc. of normal saline intravenously. The extracellular fluid was caused to decrease by the method used by Gilman (7) and Darrow and Yannet (5) which consists of the intraperitoneal injection of 5 per

cent glucose. In these two groups of experiments available fluid was calculated *1*, from a single injection of thiocyanate, determining the rate of disappearance from the percentage factor 2.3 per cent per hour, or, in some dogs, the disappearance rate was previously determined; *2*, by reinjection of thiocyanate; and

TABLE 2

Comparison of available fluid determination from original injection with available fluid as determined by reinjection of thiocyanate

EXPERIMENT	1ST EXCELL. DETN.	REINJECTION	ERROR	PER CENT ERROR
	cc.	*cc.*	*cc.*	
1	3780	3580	−200	−5.3
2	2490	2470	−20	−0.8
3	3880	3960	+80	+2.3
4	3760	3980	+220	+5.8
5	4520	4390	−130	−2.9
6	3080	3040	−40	−1.3
7	2620	2760	+140	+5.3
8	3980	4120	+140	+3.5
9	4300	4650	+250	+5.8
Average..				3.6

TABLE 3

Table illustrating changes in extracellular water in superhydration and extracellular dehydration

EXPERIMENT	PROCEDURE	ORIGINAL AVAILABLE FLUID	BY SCN DISAPPEARANCE	PER CENT CHANGE	BY SCN REINJECTION	PER CENT CHANGE	BY SODIUM METHOD	PER CENT CHANGE
1	Ureters ligated; 1000 cc. 0.9 per cent NaCl intravenously	2520	3310	+31.6	3650	+45.2	3460	+37.6
2	Ureters ligated; 1000 cc. 0.9 per cent NaCl	2870	3700	+28.6	4030	+40	4076	+42
3	400 cc. 5 per cent glucose intraperitoneally. Removal of 400 cc. fluid with 28 mgm. SCN and 1.01 grams Na	2270	1845	−18.4	1960	−13.0	2067	−9.0
4	750 cc. 5 per cent glucose IP. Removal of 840 cc. fluid with 29 mgm. SCN and 2.13 grams Na	3317	3040	−8.4	3067	−7.5	3083	−7.1
5	1000 cc. 5 per cent glucose IP. Removal of 1060 cc. fluid with 34 mgm. SCN and 2.69 grams Na	4595	4000	−13.0	3927	−14.5	4250	−5.1

3, extracellular fluid volume changes were calculated from changes in plasma sodium concentration. In the dogs receiving 5 per cent glucose intraperitoneally correction was made for the thiocyanate and sodium which diffused slowly into the peritoneal fluid. In the table it is shown that results by the three methods

are fairly concordant and are in accord with the expected type of fluid redistribution brought about by the methods used.

DISCUSSION.   According to results obtained in the present study confirmation is given to the use of the thiocyanate method in determining a partition of the body fluids as available fluid for solution of thiocyanate.   Certain precautions are necessary for proper utilization and evaluation of the test.   In order that the optimum blood level shall be produced, approximately 25 mgm. per kgm. of body weight should be administered.   In each case the diffusion time should be carefully determined by taking samples of blood, 30 minutes, one hour, one and a half hours, and two hours after the first injection.[1]   Diffusion is usually complete about one hour after the injection, but a single sample one hour after the injection is not safe because in a few dogs diffusion is not complete then. It is preferable that the disappearance rate be determined in each case by taking a sample 3 to 4 hours after the injection (before this time the decrease of thiocyanate will be too small to measure accurately) and correction for disappearance should be made in calculating the available fluid.   The use of a disappearance factor, in this study found to average 2.3 per cent per hour, yields less accurate results.   This disappearance factor is usable however, in view of the roughly quantitative nature of the test.

Determination of available fluid subsequent to the original determination may be made by one of two methods: *1*, by determining the rate of disappearance during the control period and calculating the available fluid from the thiocyanate level resulting from the original injection after changes in extracellular fluid have occurred, and *2*, by reinjection of thiocyanate.

The relatively high thiocyanate levels resulting after a second or third injection, and the possibly more rapid disappearance of thiocyanate when the plasma level is elevated cause us to favor the first method, that is, the disappearance method.   In studying changes in extracellular water it would seem preferable to check the thiocyanate method, preferably a single injection with disappearance and reinjection procedure combined, with the method of determining extracellular fluid changes by variations in sodium concentration.

The exact fate of thiocyanate was not ascertained in the present study.   The demonstrated fact that a considerably greater quantity of thiocyanate disappears from the available fluid than is excreted in the urine during a given time would strongly suggest that thiocyanate continues to enter the cells even after diffusion has been completed.

SUMMARY

1. Optimum dosage of thiocyanate to determine available fluid volume is 25 mgm. per kilogram of body weight, yielding a thiocyanate concentration of around 8 mgm. per 100 cc. of plasma.

2. The diffusion time varies but ranges around one hour.   The time should be

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[1] In recent experiments we have been giving the thiocyanate to the animal on the day preceding the experiment, taking samples at intervals on the day of injection, and the next morning taking a sample in order to calculate the rate of thiocyanate disappearance.

ascertained in each determination by serial sampling. The disappearance rate averages 2.3 per cent per hour but should also be determined in each instance and correction made accordingly.

3. Reinjection determinations of available fluid check within 6 per cent of the original determination. The disappearance method with a single injection is advocated as the preferable method for determining changes in extracellular water volume subsequent to the original determination. This method should be checked by reinjection of thiocyanate and sodium determinations.

4. In determining experimental extracellular water changes, reasonably concordant results are obtained with the thiocyanate method and the sodium method. The thiocyanate method is considered to be a satisfactory method for roughly measuring changes in extracellular water. Changes in the extracellular water volume in excess of 6 per cent probably have a definite significance by the method.

REFERENCES

(1) ASHWORTH, C. T. AND L. A. KREGEL. Arch. Surg. 44: 829, 1942.
(2) ASHWORTH, C. T., E. E. MUIRHEAD AND J. M. HILL. This Journal 136: 194, 1942.
(3) BUTLER, A. M. AND E. TUTHILL. J. Biol. Chem. 93: 171, 1931.
(4) CRANDALL, L. A., JR. AND M. X. ANDERSON. Am. J. Digest. Dis. and Nutrition 1: 126, 1934.
(5) DARROW, D. C. AND H. YANNET. J. Clin. Investigation 14: 266, 1935.
(6) ELKINGTON, J. R. AND M. TAFFEL. This Journal 138: 126, 1942.
(7) GILMAN, A. J. Clin. Investigation 14: 266, 1935.
(8) GREGERSEN, M. I. AND J. D. STEWART. This Journal 125: 142, 1939.
(9) LAVIETES, P. H., J. BOURDILLON AND K. A. KLINGHOFFER. J. Clin. Investigation 15: 261, 1936.
(10) MUIRHEAD, E. E., C. T. ASHWORTH, L. A. KREGEL AND J. M. HILL. Surgery, in press.
(11) SEYMOUR, WM. B., W. H. PRITCHARD, L. P. LONGLEY AND J. M. HAYMAN, JR. J. Clin. Investigation 21: 229, 1942.
(12) STEWART, J. D. AND G. M. ROURKE. J. Clin. Investigation 17: 413, 1938.
(13) STEWART, J. D. AND G. M. ROURKE. J. Lab. and Clin. Med. 26: 1383, 1942.
(14) STEWART, J. D. AND G. M. ROURKE. J. Clin. Investigation 21: 197, 1942.
(15) SUNDERMAN, F. W. AND F. C. DOHAN. This Journal 132: 418, 1941.

HEART RATE IN TAMED AND UNTAMED RATS

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In the course of a previous experiment (5) it was found necessary to establish the normal heart rate of the rat. This rate, it was found, depended on whether the animals were tamed or untamed. This paper presents an analysis of the differences found between the tamed and untamed rats of both sexes.

The heart rate was recorded by a Davis electroencephalograph; clip electrodes were attached ventrally and dorsally through the axis of the heart.

A miniature replica of the conventional Pavlov frame was constructed for recording the heart rate of the adult rats. The animal was kept in the frame by means of straps just behind the forelegs and in front of the hind legs. The floor of the frame is movable and serves a double purpose—it allows for adjustment to the size of the animal, and it is of aid in the training procedure. In the first few days of training, if the animal struggles too much, it displaces the floor, and consequently is left suspended by the two straps—a situation which the rat soon avoids by ceasing to struggle.

In the course of the investigations reported here, the 130 animals used were classified as follows:

1. Tamed (gentled). (a) Thirty days old. Seventeen animals in this group were handled every day from birth. They were held in the experimenter's hand while heart records were taken.

(b) One hundred-twenty days old. Twenty-seven animals in this group were handled from birth and were fastened in the Pavlov frame daily for two weeks before heart recording was begun on the 120th day.

2. Untamed (non-gentled). (a) Thirty days old. Sixty-nine animals, unhandled except for marking, comprised this group. On the 30th day heart records were taken while the animal was held in the experimenter's hands.

(b) One hundred-twenty days old. Seventeen animals, unhandled except for marking, made up this group. On the 120th day the animals were placed in the Pavlov frame and heart records taken.

Water and excess food were always available to both groups.

The two groups were thus equated with regard to the procedure employed on the 30th and 120th days, except that one group was tamed and the other untamed. In addition to the determination of heart rate, the weight of the rats was recorded. Tables 1, 2 and 3 present the results of the data analyzed on these days.

In 3 of the 4 groups the heart rate of the untamed animals was significantly higher than that of the tamed animals. That a true base line of heart rate had been reached for the tamed 120-day-old animals is indicated by the fact that the heart rate average for these 27 animals on the 120th day did not differ signif-

TABLE 1

Comparison of heart rates in tamed and untamed rats at 30 and 120 days of age

SEX	30-DAY			120-DAY		
	Tamed	Untamed	Critical ratio*	Tamed	Untamed	Critical ratio
Male	355 ± 35.6 (N:10)	496.5 ± 57 (N:32)	9.6	422 ± 30.4 (N:16)	506.7 ± 35 (N:10)	7.1
Female	413 ± 45.5 (N:7)	491.7 ± 43 (N:37)	4.2	484 ± 18 (N:11)	483 ± 44.2 (N:7)	.20

* This is an index of the reliability of a difference between 2 measures. A critical ratio of 3 or more indicates that the obtained difference is statistically significant, *i.e.*, the chances are greater than 999 in 1000 that a true difference exists.

TABLE 2

Sex differences in heart rate of tamed and untamed 30-day-old rats and of tamed and untamed 120-day-old rats

AGE	HEART RATE					
	Tamed group			Untamed group		
	Male	Female	Critical ratio	Male	Female	Critical ratio
30 days	355 ± 35.6 (N:10)	413.2 ± 45.5 (N:7)	2.8	496.5 ± 47.1 (N:32)	491.7 ± 43 (N:37)	.4
120 days	422 ± 30.4 (N:16)	484.2 ± 18 (N:11)	6.7	506.7 ± 34.8 (N:10)	483 ± 44.2 (N:7)	1.2

TABLE 3

(a) *Differences in weight of tamed and untamed 30-day-old animals and tamed and untamed 120-day-old animals (grams)*

SEX	30-DAY			120-DAY		
	Tamed (1)	Untamed (2)	Critical ratio	Tamed (3)	Untamed (4)	Critical ratio
Male	41.3 ± 8.5 (N:10)	51.1 ± 10.5 (N:32)	2.9	265.7 ± 23.2 (N:16)	303.5 ± 23.1 (N:10)	4.2
Female	43.2 ± 6.6 (N:7)	49.8 ± 11.2 (N:37)	2.0	202.5 ± 13.4 (N:11)	229.4 ± 12.2 (N:7)	4.3

(b) Critical ratio sex differences in wt. col. 1 = 0.5 (not significant).
Critical ratio sex differences in wt. col. 2 = 0.5 (not significant).
Critical ratio sex differences in wt. col. 3 = 9.0 (significant).
Critical ratio sex differences in wt. col. 4 = 8.6 (significant).

icantly from the average heart rate of these same animals based on recordings taken from the 120th to the 134th day inclusive. These rates were 447 for the

1 day period and 451 for the total 15 day period. The procedure of taming outlined above then allows for a reliable 1 day sampling.

The untamed females were less affected by the recording situation than were the untamed males.

Sex differences are present in the tamed animals but not in the untamed animals. Sex differences, however, disappear in the tamed group when recordings are taken under conditions of stress. The latter point was illustrated when the tamed animals of 120 days of age which showed a sex difference with a critical ratio of 6.7 were reintroduced to the Pavlovian frame after a 50-day absence. The situation now, judging by struggling behavior and defecation, was a stressful one and analysis of the data of recordings taken for a 5-day period failed to reveal any sex difference. The rates of both sexes were approximately 475. This disappearance of sex differences may be said to be due mainly to the different action of the male and female heart under stress, the former reaching a higher rate while the females apparently were unaffected. If male and female heart rate gained proportionately, sex differences would still be observable. The fact that they do not results in the males reaching a level approximately equal to that of the females with a consequent disappearance of previously noted sex differences. It was also noted (table 1) that while both untamed male age groups showed a significant difference from the corresponding tamed animals only one of the two female groups showed that difference. These two facts then might suggest a sex difference in regard to sensitivity of heart rate.

Fluctuation in heart rate. This was calculated by analyzing the 30-second period into five 6-second periods and considering the maximum change between any two periods as the fluctuation. No significant differences were found in any of the cross comparisons.

In three of the four comparisons the untamed rats were significantly heavier than tamed rats of the same age and sex. Sex differences in weight were proportional in the tamed and untamed animals for the two age groups.

DISCUSSION. Hammett (2) in 1921 reported, in this Journal, on the mortality rate of two groups of rats in the 48 hours following thyroparathyroidectomy. For the "standard rats" the mortality was 79 per cent of a group of 90; for the "experimental rats," 13 per cent of a group of 96. The "standard rats" referred to the stock albinos, animals rarely handled up till the time of operation. The "experimental rats" were described as animals which had had constant handling and petting. Hammett's "standard rats" then correspond to the untamed rats of the present study, and his "experimental rats," to the tamed rats. Hammett suggests the following hypothesis to account for the difference in mortality rate. The excitement of the standard rats on being handled led to a high muscular tone with a resulting high metabolic rate and more rapid formation of toxic compounds. The experimental rats, on the other hand, maintained a low muscular tone and lower metabolic rate and a less rapid rate of toxin formation. As a result the experimental (tamed) animals survived in larger numbers. The findings of the present study showing differences in heart rate between tamed (experimental) and untamed (standard) animals supplement and support

Hammett's hypothesis, the higher heart rate of the untamed animal implying a higher metabolic rate. This differentiation in heart rate between tamed and untamed rats may also have some bearing on the findings of Humphrey and Marcuse (3) that the untamed rats are more susceptible to convulsive seizures due to auditory stimulation.

Sex differences in metabolic rate, weight of hypophysis, blood coagulation time, etc., have been found in the rat (1). Thus it is not surprising that sex differences in heart rate should exist. The lack of recognition of this difference in the available literature on the subject may be a result of the masking effect on this difference caused by the differential reaction of male and female heart to conditions of stress implied in the recording situation. Schafer (6) states that while the pulse frequently is greater in women than in men, this difference almost disappears if men and women of equal stature are compared. In the present investigation sex differences in weight were present in both the tamed and untamed 120-day-old group. The differences were proportional for the two groups with the males being the heavier. Sex differences in heart rate, however, were found only in the tamed group. In the 30-day group sex differences in weight were not present for either the tamed or untamed groups, but again sex differences in heart rate were found for the tamed group only (tables 2 and 3b). Weight then does not appear to be significantly related to heart rate. This finding, together with the unusual sequence of development of heart rate found in the rat, *low* at birth with subsequent increase, (4), may suggest an atypical heart action system.

SUMMARY

1. Heart rates of untamed 30- and 120-day-old rats were significantly higher than heart rates of tamed rats of the same sex and age in three of four comparisons. This higher rate was more marked in the males.

2. Sex differences noted in the tamed group were not present in the untamed group or in the tamed group when under stress.

3. Untamed animals were significantly heavier than tamed animals of the same sex and age in three of four comparisons.

4. No significant differences in fluctuation of heart rate were found in any cross comparison of groups.

REFERENCES

(1) GRIFFITH, J. Q. AND E. J. FARRIS (editors). The rat in laboratory investigation. Philadelphia, J. B. Lippincott, 1942, p. 488.
(2) HAMMETT, F. S. This Journal 56: 196, 1921.
(3) HUMPHREY, G. AND F. MARCUSE. J. Comp. Psychol. 32: 285, 1941.
(4) MARCUSE, F. L. AND A. U. MOORE. This Journal 139: 49, 1943.
(5) MARCUSE, F. L. AND A. U. MOORE. J. Exper. Psychol., in press.
(6) SCHAFER, E. A. Textbook of physiology. Vol. 2. New York, The Macmillan Co., 1900, p. 1362.

DEMONSTRATION OF ANTITHROMBOPLASTIC ACTIVITY IN NORMAL AND HEMOPHILIC PLASMAS

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The study of blood clotting has concerned itself to a large extent with the transformation of prothrombin into thrombin and of fibrinogen into fibrin. This emphasis, on what must constitute, under ordinary conditions, the later phases of the changes in the blood after it leaves the vessels has been encouraged by the view that normal "cell free" plasma contains all factors needed for coagulation (1). Though this may be true, the point is often overlooked that the *time* at which coagulation begins and the *rate* of its development are directly influenced by the degree and extent of the changes in shed blood *preceding* the inception of clotting.

It is generally agreed that a clot accelerating substance, thromboplastin, released from tissue cells or the formed elements of shed blood is responsible, in a large measure, for the rapid activation of prothrombin. The mechanism whereby prothrombin is activated remains obscure; the rate and extent of activation is somehow related to the amount of thromboplastin available (2, 3, 4). The following experiments may help to throw additional light on this mechanism. The evidence to be presented appears to indicate that normal human "cell free" plasma *collected with especial precautions*, has the property of reducing the clot accelerating action of aqueous extracts of brain tissue, when plasma and extract are incubated before recalcification. This natural clot decelerating quality of fresh human plasma shall be designated "antithromboplastin activity," or merely "antithromboplastin." Hemophilic plasma possesses an antithromboplastin activity several times greater than normal. This excess is probably the *primary* cause of the pronounced delay in the inception of coagulation of hemophilic blood.

METHODS AND REAGENTS. Unless meticulous care is taken in the collection of blood and separation of plasma little or no activity may be demonstrated. For this reason the various technical steps will be described in detail. Venous blood is collected, preferably from a fasting subject, swiftly (average rate of collection no less than 1 cc. each 2 sec.) through sharp, wetted 18 gauge needles, into syringes loaded with the required amount of anticoagulant. All bubbling should be avoided, and the blood should be gently mixed with the anticoagulant within 30 sec. after it began to be drawn into the syringe. The blood is centrifuged immediately at 3,000 RPM for 45 minutes, the upper $\frac{2}{3}$ of the plasma carefully removed *at once* with paraffin coated droppers, placed in similarly coated tubes, and tested within one hour after its separation. Occasionally, in blood with a high per cent cell volume, centrifugation for 45 min. at 3,000 RPM

does not suffice to rid the plasma of platelets. As additional precaution, a platelet count (1–20 dilution) on the plasma should be done, to make certain that it does not contain more than 4,000 true platelets per mm³. A 0.129 M sodium citrate solution (3.8 grams of $Na_3C_6H_5O_72H_2O$ in 100 cc. distilled H_2O) is used as anticoagulant in the proportion of 1 part of the solution to 9 parts of blood, when the per cent cell volume is 40 ± 5. This will yield a plasma with a citrate concentration between 0.0187 and 0.0214 M (0.55 and 0.63 gram per cent). Recalcification of 0.3 cc. of such plasma (containing 1.6 to 1.9 mgm. of citrate) with 0.1 cc. of a solution containing between 0.7 and 1.0 mgm. of $CaCl_2$ will yield, fairly constantly, optimal (minimal) coagulation times. Care must be taken, however, that the proportions of citrate to calcium are not greatly upset by wide variations in the per cent plasma volume in a given specimen. In order to maintain uniform citrate-calcium ratios when standard calcium solutions are used for recalcification, one must *add* to the blood 0.16 cc. of the citrate solution for every 10 units (or fraction) *below* 35, of the red cell hematocrit; a similar amount should be subtracted from 1 cc. of the citrate solution to be added to the blood, for each 10 units (or fraction) of the red cell hematocrit *above* 45. If the per cent cell volume has not been determined in advance, the amount of $CaCl_2$ required to give a minimal coagulation time for a given mixture of plasma and diluted thromboplastin must be established by trial. As will be shown later, 0.1 cc. of a $CaCl_2$ solution between 0.06 and 0.09 M will nearly always produce minimal clotting times when added to 0.3 cc. of plasma of 0.017–0.022 M citrate concentration (0.5 to 0.65 gram per cent).

The following standard thromboplastin solutions are used: 1. *Concentrated:* 0.85 per cent NaCl extract of acetone dried adult human brain prepared by the Quick method (5). Each 0.1 cc. of this solution should clot 0.1 cc. of normal human recalcified citrated plasma in 11–12 sec.; this time should not be perceptibly altered by incubation of solution with plasma, in these proportions, before recalcification. 2. *Dilute* thromboplastin solutions. *Solution A:* prepared by diluting 0.025 cc. of the concentrated saline brain extract, measured in a serological pipette, with 10 cc. of 0.85 per cent NaCl. Each 0.1 cc. of this solution contains 0.25 mm.³ of the concentrated thromboplastin and should clot 0.3 cc. of optimally recalcified normal citrated human plasma in 50 ± 10 sec. *Solution B:* prepared by diluting 0.033 cc. of a 1–10 dilution of the concentrated saline brain extract with 10 cc. of 0.85 per cent NaCl. Each 0.1 cc. of this solution contains 0.033 mm.³ of the concentrated thromboplastin and should clot 0.3 cc. of optimally recalcified normal, citrated, human plasma in 100 ± 10 sec. The dilute solutions should be fresh; they begin to lose potency after about 2 hours' standing at room temperature. Recalcification is carried out with 0.1 cc. of a $CaCl_2$ solution of varying molar concentration, according to the respective *total* amount of citrated plasma used. If a minimal clotting time on 0.3 cc. of plasma is obtained with 0.1 cc. of a $CaCl_2$ solution other than 0.074 M, the concentration of the $CaCl_2$ for other quantities of plasma must be correspondingly adjusted.

The amounts and order of addition of the reagents are as follows: *Tube 1*

(without incubation): 0.1 cc. diluted thromboplastin, 0.1 cc. $CaCl_2$, 0.3 cc. plasma. *Tube 2* (with incubation): 0.1 cc. diluted thromboplastin, 0.3 cc. plasma, mix, incubate, then add 0.1 cc. $CaCl_2$.

When the effect of a certain agent (heat, dilution, etc.) is being studied, the distribution of amounts and order of addition are as follows: *Tube 1* (without incubation): 0.2 cc. *treated* plasma, 0.1 cc. *fresh* plasma, then rapidly 0.1 cc. $CaCl_2$ (proper M concentration) and 0.1 cc. diluted thromboplastin. *Tube 2* (with incubation): 0.2 cc. *treated* plasma, 0.1 cc. diluted thromboplastin, mix, incubate, then *rapidly* 0.1 cc. $CaCl_2$ (proper molar concentration), and 0.1 cc. *fresh* plasma.

In control tubes the treated plasma is replaced by an equal volume of 0.85 per cent NaCl, the order of additions and rest of procedure remaining the same. In this manner the clotting system $CaCl_2$-fresh plasma is used as an "indicator" of the extent to which incubation with the *treated* plasma (or salt solution) has affected the clot accelerating power of the thromboplastin. The order of additions is so arranged as to allow no opportunity for contact of the thromboplastin with the plasma before recalcification, except when so designed.

Prothrombin is measured by Quick's method (5) using the acetone dried human brain extract. Unless otherwise stated the prothrombin content of all plasmas is 100 per cent of normal. The rate of clotting of plain recalcified citrated plasma (designated throughout these experiments as the "Plasma Clotting Time") is estimated by adding 0.1 cc.of $CaCl_2$ (proper M concentration) to 0.3 cc. of plasma. All incubations and clotting time determinations unless otherwise stated are carried out at $37.5 \pm 0.5°C$. in glass tubes 7 mm. internal diameter and 3 cm. in length. The evidence in support of the conclusions in this paper was selected from a group of 351 experiments on blood from dogs, rabbits and men (normal and hemophilic).

1. *Incubation of plasma with thromboplastin solutions.* A diminution in the clot accelerating action of certain thromboplastin solutions may be observed as early as after one minute of incubation with normal plasma (fig. 1). A clear antithromboplastin effect is observed only with solutions of a given clot accelerating power (those containing 1.0 mm.³, 0.25 mm.³ or 0.033 mm.³ of concentrated thromboplastin per 0.1 cc.). A maximum effect seems to be reached after about 5 min.; between that time and 10 min. no further important changes seem to take place. Hemophilic plasma differs from normal in that: 1, it exerts a clot decelerating effect even on potent thromboplastin solutions (10 mm.³/cc.); 2, its effect on the same solution is more pronounced, and 3, within certain limits, grows more intense as the solution becomes weaker.

Since most of the inactivation of thromboplastin by plasma apparently takes place in the first 3 min. of incubation it would be natural to expect that, when the thromboplastin is so diluted as to produce a clotting time approximating that of recalcified plasma without thromboplastin, little or no difference would be shown between the incubated and unincubated specimens. In such instances, the coagulation of the recalcified unincubated plasma itself proceeds at a rate sufficiently slow, to allow most or all of the inactivation to take place during

that period; preliminary incubation with the thromboplastin for 5 min. will, therefore, result in little addition to the amount of thromboplastin inactivated. A similar effect is observed when coagulation of normal plasma is made more prolonged by increasing the *volume* of the plasma while holding the volumes of thromboplastin and CaCl₂ fixed. When the clotting times goes beyond 120 sec. the antithromboplastin effect gradually diminishes until a point is reached when the incubated specimens clot faster than the unincubated. In order to
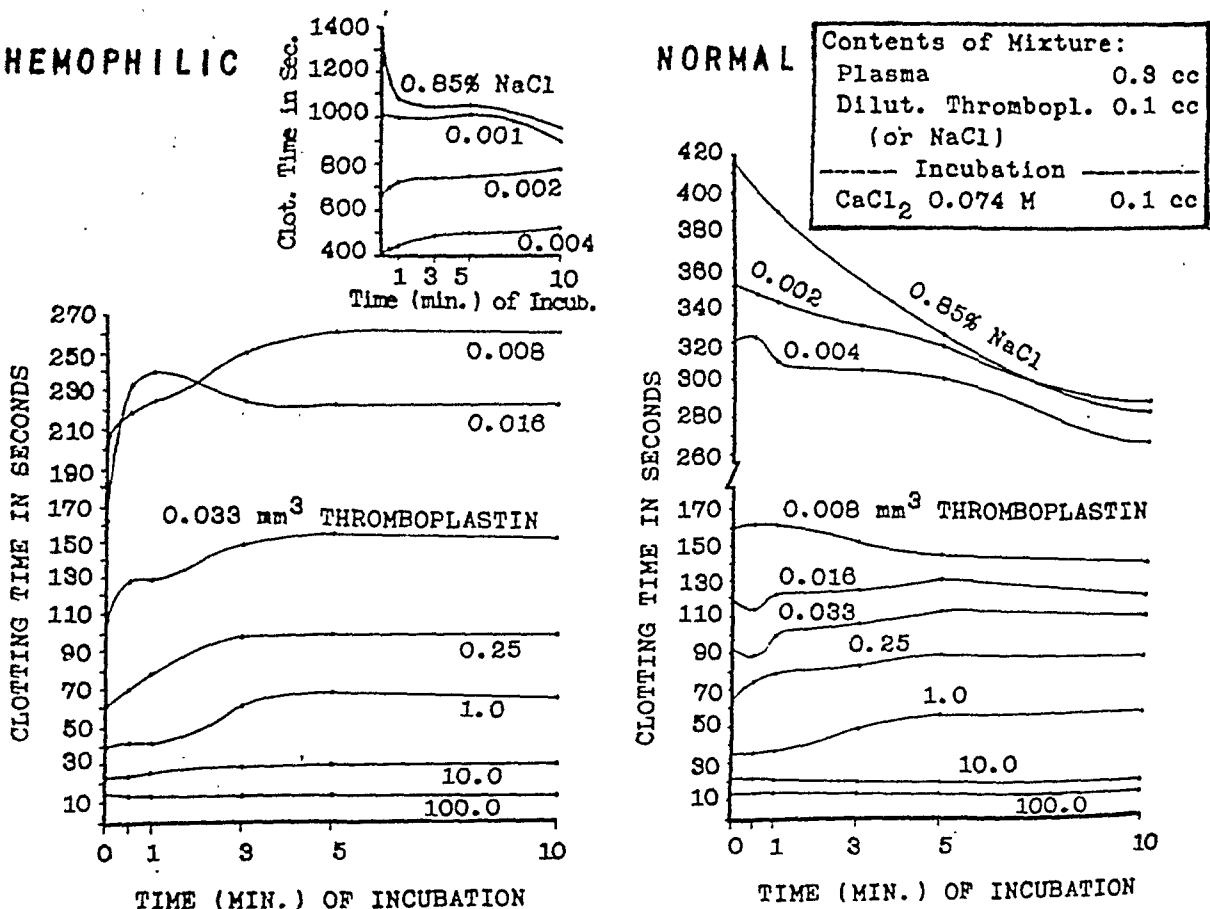
Fig. 1. Effect on the clotting time of hemophilic and normal plasma of incubation for various periods with solutions of thromboplastin of decreasing potency, before recalcification. The numbers adjacent to each curve designate the potency of the thromboplastin solution used, expressed in terms of the actual amount in cubic millimeters of concentrated saline brain extract in each 0.1 cc. of the solution.

detect, by the incubation method, maximum decelerating effects in normal plasma, a thromboplastin solution that produces a clotting time of about 50 sec. seems best. For comparing the activity of normal plasma with that of hemophilics a clotting time of about 100 sec. is desirable. This presupposes equal prothrombin concentrations in the 2 plasmas. "Cell free" oxalated plasmas, separated from blood colllected as specified, behave in this respect substantially like citrated plasmas.

In table 1 are analyzed statistically the extent of the clot decelerating action

of the "cell free" plasmas of 51 normal and 6 hemophilic men on the dilute thromboplastin solutions A and B. When using the stronger thromboplastin (sol. A), one may expect in 68.2 per cent of determinations on normal subjects, prolongations of the clotting time of the incubated plasma above that of unincubated, of the order of 12.5 to 43.1 sec.; an equivalent percentage of determinations on hemophilic plasma should yield prolongations of 32 to 55.6 sec. When using the weaker thromboplastin (sol. B) a prolongation of the order of 8 to 29.2 sec. only may be expected in normal plasma, while hemophilic plasmas may yield prolongations of 49.6 to 71.7 sec. As indicated by the values of p, hemophilic plasma exerts an effect significantly greater than that exerted by normal plasma against the weaker thromboplastin solution; against the stronger solution, no statistically significant difference exists between the 2 plasmas.

The longer coagulation time of the incubated plasma-thromboplastin mixture is not due to a diminution in prothrombin, since no difference may be observed

TABLE 1

Significance of differences in antithromboplastin activity between normal and hemophilic plasmas, using 2 types of thromboplastin

TYPE OF THROM-BOPLASTIN	NORMAL INCREASE IN CLOT. TIME OF INCUB. OVER UNINCUB.			HEMOPHILIC INCREASE IN CLOT. TIME OF INCUB. OVER UNINCUB.			DIFFER. ± σ DIFF.	$\frac{\bar{x}}{\sigma\text{DIFF.}}$	p^*
	Mean (±σ)	Max. Min.	No. of observ.	Mean (±σ)	Max. Min.	No. of observ.			
	sec.			*sec.*			*sec.*		*per cent*
Sol. A	27.8 ±15.3	44 13	59	43.8 ±11.8	92 23	29	16 ±19.3	.82	41
Sol. B	18.6 ±10.6	45 1	38	60.7 ±11.09	114 40	35	42.1 ±15.29	2.75	0.6

* Probability of the difference between the 2 plasmas being due to sampling.

between the prothrombin content of unincubated plasmas and those incubated with dilute thromboplastin solutions for the periods of time stated above. Likewise, incubation of the plasma and thromboplastin solution separately, or of the plasma with 0.85 per cent NaCl before recalcification, either does not alter the clotting time significantly or shortens it, when compared with unincubated controls. Moreover, if the thromboplastin solution is pipetted down carefully on the surface of the plasma and the two are allowed to incubate, without mixing, much less prolongation of the clotting time is observed than when the thromboplastin and plasma are mixed before incubation (table 2). Restricting the site of the reaction between the two liquids to their interface, accounts perhaps for the diminished effect.

2. *Calcium and antithromboplastin.* The importance of maintaining uniform citrate/calcium ratios in these studies may be appreciated when the effect of various amounts of sodium citrate and $CaCl_2$ on antithromboplastin activity is studied.

Normal or hemophilic blood (9 cc.), collected as specified, was drawn into 2 syringes loaded with 1 cc. of a solution of either 0.064 M (1.9 per cent) sodium citrate in 0.4 per cent NaCl or 0.129 M (3.8 per cent) sodium citrate. The bloods were then centrifuged at 3,000 RPM for 45 min. and the respective amount of citrate in each plasma calculated from the percent cell volume. One sample of blood was allowed to stand at 5°C. for 24 hrs. before the plasma was separated by centrifugation. The activity of the normal plasmas was tested on thromboplastin solution A, that of hemophilic plasmas on sol. B, using $CaCl_2$ solutions of varying molar concentration.

With 0.02 M citrate plasmas, minimal clotting times and an antithromboplastin activity of uniform degree are obtained on recalcification with 0.06–0.074 M $CaCl_2$ solutions; under-citrated, 0.01 M plasmas may not manifest a comparable degree of activity when these $CaCl_2$ solutions are used (table 3). Over-recalcification of the plasma, regardless of its citrate content, prolongs the clotting time and gradually abolishes all evidences of antithromboplastin activity. Under-recalcification also prolongs the clotting time, but greatly accentuates

TABLE 2

Effect on clotting time of a mixture of 0.3 cc. plasma, 0.1 cc. diluted thromboplastin and 0.1 cc. 0.074 M $CaCl_2$, of incubation (5 minutes) of the thromboplastin or plasma in various ways, before recalcification

PLASMA SPEC. NO.	WITHOUT INCUB.	THROMBOPL. AND PLASMA MIXED AND INCUBATED TOGETHER	THROMBOPL. AND PLASMA INCUBATED TOGETHER, NOT MIXED	THROMBOPL. AND PLASMA INCUBATED SEPARATELY	PLASMA ALONE INCUBATED	THROMBOPL. ALONE INCUBATD
			Clotting time in seconds			
1	46	72	53	48	46	46
2	95	139	107	96	92	94

the antithromboplastin effect. Similar trends, though on a larger scale, are observed with hemophilic plasma. In contrast, when the 24 hr. old normal plasma, which had lost most of its antithromboplastin, was tested, the difference between incubated and unincubated specimens was slight. The 24 hr. old hemophilic plasma behaved essentially as a fresh specimen of normal plasma.

The slowing of the rate of prothrombin transformation resulting from an insufficient amount of calcium probably entails a slower utilization of any available thromboplastin. The effect of such a slowing would in turn be magnified by any reduction in potency of the thromboplastin, following incubation with plasma. An exaggeration of the antithromboplastin effect might then be properly expected to occur. Attention has been drawn by Minot and Lee (6) to the relatively greater clot delaying effect of under-recalcification on hemophilic plasma than on normal plasma. The mechanism underlying the elimination of the antithromboplastin effect by addition to the plasma of calcium in excess of that required for optimum coagulation, is not clear.

3. *Platelets and antithromboplastin.* Plasma of different platelet content was obtained by centrifugation of citrated blood for 1, 10 and 45 min., respectively,

at 3,000 RPM. The clotting time of the plain recalcified plasmas and the antithromboplastin effect of each on 2 solutions of thromboplastin were then measured. As shown in table 4, the plasma clotting time becomes shorter and the

TABLE 3

Effect of various amounts of calcium on clotting time and antithromboplastin activity of "cell free" plasma of different citrate content

0.3 cc. plasma, 0.1 cc. thromboplastin (0 minute or 5 minutes incubation), 0.1 cc. $CaCl_2$ solution

TYPE OF PLAS.	CITR. IN PLAS.	INCUBA-TION	MOLAR CONCENTRATION $CaCl_2$								
			0.027	0.045	0.06	0.074	0.091	0.118	0.14	0.18	0.22
		min.	*Clotting time in seconds*								
Normal, fresh	0.01 M	0	59	53	50	59	73	99	103	167	
		5	126	111	76	78	87	103	100	145	
	0.02 M	0	480	62	56	50	64	89	93	181	
		5	660	103	90	81	88	98	105	117	
Normal 24 hr. old	0.02 M	0	170	52	39	31	34	40	43	48	50
		5	184	63	48	38	34	39	42	42	43
Hemoph., fresh	0.01 M	0	156	122	116	116	143	175	277	348	514
		5	232	181	172	178	179	197	237	275	330
	0.02 M	0	412	121	113	113	123	141	150	198	274
		5	1216	190	182	171	170	184	187	227	237
Hemoph., 24 hr. old	0.02 M	0	439	117	108	104	110	115	126	150	215
		5	626	165	147	132	130	133	136	140	154

TABLE 4

Effect of number of platelets on antithromboplastin activity of a specimen of normal human, citrated plasma

PLASMA SPEC. NO.	NO. OF PLATELETS	CLOT. TIME OF PLASMA + DIL. THROMBOPLASTIN*				CLOT TIME OF PLASMA ALONE†
		Sol. A		Sol. B		
		0 minute incub.	5 minute incub.	0 minute incub.	5 minute incub.	
	thous./c. mm.	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*
1	4	67	103	97	118	390
2	114	72	88	99	104	195
3	420	67	76	96	97	150

* 0.3 cc. plasma, 0.1 cc. dil. thrombopl. sol., 0.1 cc. 0.074 M $CaCl_2$.

† 0.3 cc. plasma, 0.1 cc. 0.074 M $CaCl_2$.

antithromboplastin effect diminishes, as the number of platelets increases. This is especially evident with the weaker solution of thromboplastin. Plasma containing intact platelets is, therefore, unsuitable for these experiments. Although

during the incubation there is some inactivation of the added thromboplastin, upon recalcification fresh thromboplastin is probably released from the platelets, thereby offsetting to a variable extent the inactivating effect of the incubation. This uncertainty as to the amount of thromboplastin derived from disintegrating platelets renders saline or water extracts of washed human platelets likewise unsuitable as a source of this material, instead of dilute brain extracts. Theoretically, platelets should constitute the best source of thromboplastin for this purpose, since the action of antithromboplastin in the circulating blood is probably exerted mainly on the products of platelet disintegration. Nevertheless, suspensions of washed human platelets, alcohol, ether and distilled water extracts of platelets, frozen and thawed distilled water platelet extracts have, so
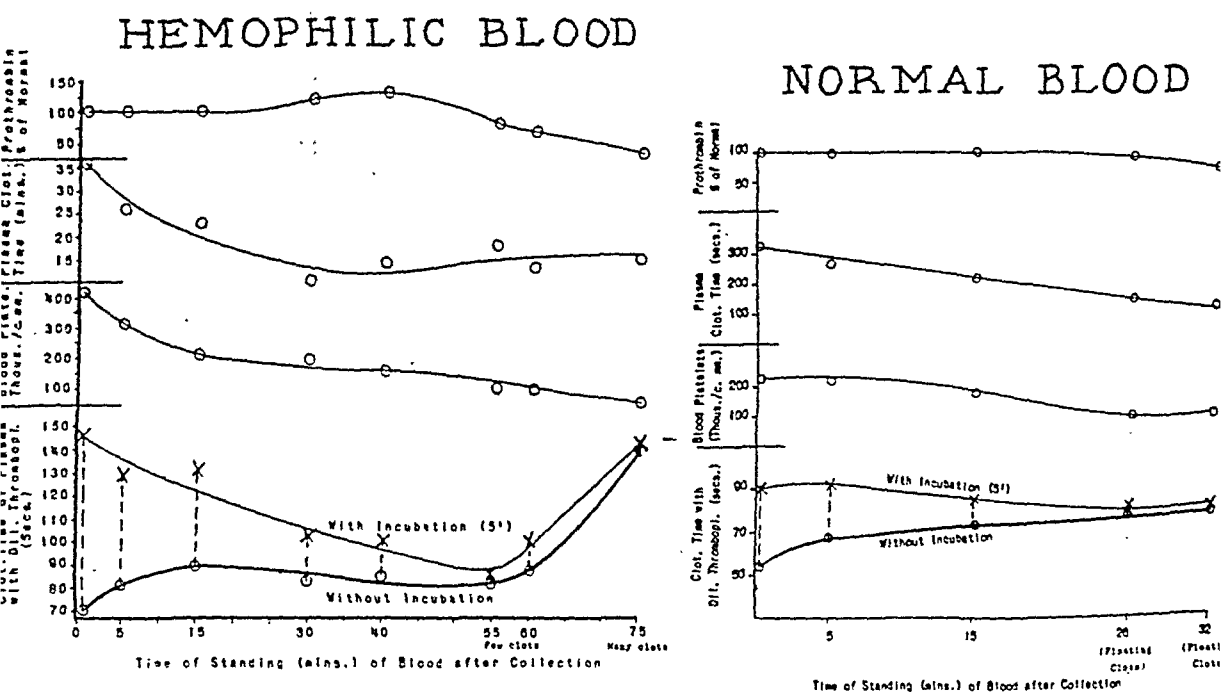
Fig. 2. Changes in prothrombin, platelets, plasma clotting time and antithromboplastin in standing hemophilic and normal blood.

far, in our hands, proved unsuitable as sources of thromboplastin for these experiments. With none of these extracts has it been possible to reduce the coagulation time of 0.3 cc. recalcified citrated plasma to 50 sec. or less.

If plasma is allowed to stand in contact with formed elements of the blood, its antithromboplastin is gradually reduced until no trace of it may be found. A diminution is detected within the first 90 min. of standing (table 5). At the end of the 6 hr. period, when over half the number of platelets has disintegrated, no more activity can be detected. Little change in prothrombin takes place during this interval. As antithromboplastin diminishes and more platelets are destroyed, the plasma clotting time becomes shorter. If the plasma is kept in a paraffin coated tube, the changes may be somewhat delayed. Nearly always, however, no activity may be found after a 24 hr. period of standing. Platelet rich hemophilic plasma may require more than 24 hrs. before all its activity

disappears; platelet free hemophilic plasma may maintain some of it for as long as 8 days. There is likewise a significant diminution in antithromboplastin and in the clotting time of plasma separated from venous blood collected with difficulty or that obtained by an incision of the skin. Transient exposure to tissue juices usually neutralizes all the antithromboplastin in normal plasma, and reduces it somewhat in hemophilic plasma.

The greater coagulability of plasma after standing in contact with formed elements, or with injured tissues must therefore be attributed not only to an increased content of *free* thromboplastin, but also to the diminution of antithromboplastin. These results emphasize the importance of rapidity and thoroughness in collecting the blood and in separating the plasma from its cellular elements, for assurance, before testing the plasma, that there is no cell-bound thromboplastin in it.

4. *Fate of antithromboplastin in shed normal and hemophilic blood.* Twenty five cubic centimeters of normal or hemophilic blood were collected rapidly into

TABLE 5

Changes in number of platelets, clotting time of the plasma, prothrombin time and antithromboplastin in a sample of citrated platelet-rich plasma, standing in a glass tube at 20°C. before being centrifuged and tested

	TIME OF STANDING (MIN.)				
	0	90	180	270	360
No. of platelets (thous./c.mm.)	420	410	340	202	180
Plasma clotting time (sec.)	320	267	235	150	130
Prothrombin time (sec.)	12.4	12.5	12.7	12.5	13.5
Plas. + sol. A + CaCl₂					
0 minute incubation (sec.)	65	69	73	77	65
5 minutes incubation (sec.)	95	93	85	82	62

an oiled syringe, without anticoagulants, and allowed to stand in a covered paraffin coated tube at 20°C. At the intervals shown in figure 2, 2.7 cc. of blood were removed from the tube into 0.3 cc. of a 0.129 M sodium citrate solution. After counting the free platelets, the mixture was centrifuged for 45 min. at 3,000 RPM and the plasma examined. Normal blood standing *under the specified conditions* shows little change in the first 5 min. Between 5 and 15 min. there is a diminution in the number of platelets and in antithromboplastin, accompanied by an increase in free thromboplastin as indicated by shortening of the plasma clotting time. For the next 10 min. these changes become even more pronounced, until no significant amount of antithromboplastin can be detected and gross clots begin to appear. The prothrombin, which up to this time had been unchanged, begins to decline, and coincident with it, massive clotting occurs. In a specimen (not shown in the figure) collected at 35 min., and consisting principally of serum, the prothrombin was 7 per cent of normal, the platelets 20,000 per c.mm., the plasma clotting time 440 sec. (thready clot) and there was no trace of antithromboplastin. As a contrast, in hemophilic

blood, only after 55 min. have elapsed and proportionately more platelets have been destroyed, is a pronounced decrease in antithromboplastin observed. Then, clots appear, the prothrombin begins to decline and the plasma clotting time shortens.

It appears, therefore, that in blood shed without contact with tissue juices or water wettable surfaces, actual *coagulation* (formation of thrombin and fibrin) occurs relatively late, though it proceeds rapidly once it has begun. This fact is supported by the knowledge that no significant change occurs in the viscosity of shed blood and no fibrin may be observed in it under the ultramicroscope until after several minutes of standing (7, 8). Most of the time interval between collection of blood and appearance of clots appears to be taken up with the slow release of thromboplastin from disintegrating platelets, and its inactivation by antithromboplastin. After the latter is depleted, any thromboplastin subsequently released from the remaining platelets (or leukocytes) apparently acts directly on prothrombin. These observations make it seem unlikely that free thromboplastin exists, as proposed (13, 10), in the circulating blood. If such were the case the activation of prothrombin should begin as soon as the blood is shed.

5. *Effect of dilution.* Fresh, normal or hemophilic "platelet free" plasma was diluted 1–2, 1–4, etc. with 0.85 per cent NaCl, and the activity of 0.2 cc. of each dilution tested on either sol. A or B by the methods described. The tests must be run *immediately* after each dilution is made, as diluted plasma loses its antithromboplastin potency rapidly on standing. Dilution diminishes the ability of the plasma to reduce the clot accelerating action of thromboplastin (table 6) until a point is reached when the difference between the clotting times of incubated and unincubated plasmas is not materially greater than that between the incubated and unincubated controls (0.85 per cent NaCl and thromboplastin). A test of the thromboplastin inactivating effect of serial dilutions of a given plasma might, therefore, be used as a measure of its antithromboplastin activity. Such activity may be expressed in terms of the smallest amount of plasma which, when incubated with a given volume of a standard solution of thromboplastin, will significantly reduce its clot accelerating action. A significant reduction is considered to exist when such incubation raises the clotting time of the diluted plasma 5 or more sec. above that of a control (thromboplastin solution incubated with 0.85 per cent NaCl). The minimum amount of *normal* plasma required to yield a clear inhibitory effect on 0.1 cc. of sol. A is fairly constant: 0.013 cc., or a 1–15 dilution of normal plasma; only 0.006–0.005 cc. of *hemophilic* plasma (or 1–30 to 1–40 dilution) is needed to produce the same effect. Against the weaker solution of thromboplastin (solution B), greater and more widely divergent amounts of normal plasma are required: 0.1 to 0.033 cc., (1–2, 1–6 dilution) while only 0.006–0.004 cc. of hemophilic plasma are necessary. These findings seem to indicate a clot decelerating activity in hemophilic plasma at least 5 to 8 times greater than normal against thromboplastin solutions producing a clotting time of about 100 sec. In any statement regarding degree of antithromboplastin activity it is important to specify the clotting time of the mixture in which the activity is being tested. While hemophilic plasma main-

TABLE 6

Effect of incubating 0.2 cc. of various dilutions of plasma from 5 subjects (3 hemophilic and 2 normal) with 0.1 cc. of either of 2 thromboplastin solutions, before recalcification and addition of 0.1 cc. of plasma

AMOUNT OF PLASMA IN 0.2 CC.	INCUB. PERIOD	SUBJECT									
		S. M. (hemo.)		N. F. (hemo.)		D. W. (hemo.)		R. B. (Norm.)		J. L. (Norm.)	
		Type of thromboplastin solution									
		A	B	A	B	A	B	A	B	A	B
	min.	Clotting time in seconds									
0.2	0	54	98	61	112	69	112	54	114	57	106
	5	93	141	100	159	110	162	76	136	76	126
0.10	0	45	96			63	109		117	53	102
	5	81	140			106	142		128	64	123
0.05	0	47	96						114	54	99
	5	69	134						116	62	120
0.033	0	45						52	111	53	104
	5	67						68	111	62	115
0.02	0	45	106	60	88			48		51	98
	5	65	136	91	117			61		60	104
0.013	0	46	106					48		50	
	5	64	130					56		59	
0.01	0	46	106	62	97	62	109	49		51	
	5	61	128	84	119	83	131	53		51	
0.0065	0	49	110		95	61	110	49		50	
	5	59	128		111	70	126	49		52	
0.005	0	52	109	64	91	58	111	50		51	
	5	60	116	71	106	66	116	48		51	
0.004	0	51	108	65	92	64	112				
	5	55	110	69	103	64	114				
0.0033	0	53	113		91						
	5	55	110		98						
0.85% NaCl	0	53	108	66	90	60	112	47	111	50	102
	5	54	106	65	96	63	115	49	115	51	103
Antithrombopl. Titer* (cc.)		0.005	0.005	0.005	0.004	0.006	0.006	0.013	0.10	0.013	0.033
Plas. clotting time (sec.)		2100		3060		3300		370		354	

* Minimal amount of plasma capable of exerting a significant antithromboplastin effect.

tains, and even increases its activity as the clotting time of the mixture becomes longer (fig. 1) it is not possible to demonstrate this property in normal plasma when the clotting time reaches 130 sec. or over. A convenient point for comparison is that when the 2 unincubated plasmas clot at approximately the same time, with a thromboplastin which is not potent enough to overwhelm the inhibitors. A measure of activity at such levels may, however, apply only to comparisons between hemophilic and normal plasmas. For a comparison of antithromboplastin content of hemophilic plasmas from various subjects, slower acting thromboplastins are desirable.

6. *Effect of heating.* Temperatures of 45° or 50°C. for 5 min. impair somewhat the clot decelerating activity of plasma. Greater impairment follows heating at 55°, and at 65° all activity is destroyed. Antithromboplastin seems to be slightly more heat stable than prothrombin (which is almost entirely destroyed at 55°C.), and not as stable as antithrombin (which resists 60° for 10 min. without much loss in activity). This and the fact that antithromboplastin is almost absent from the blood when thrombin formation begins, makes it seem that the natural antithromboplastin of the plasma is entirely distinct from the natural antithrombin. Another indication of the close specificity of the reaction between thromboplastin and antithromboplastin is obtained by noting the effect of heating the thromboplastin. Temperatures of 100°C. (15 mins.) not only impair its clot accelerating power but almost abolish its susceptibility to antithromboplastin.

7. *Species specificity.* For a clear demonstration of antithromboplastin activity, the brain extracts used as sources of thromboplastin must come from animals of the same species as the plasma being tested. The greatest clot decelerating effect is observed when human thromboplastin is incubated with human plasma; a significant though not as great an effect takes place when rabbit thromboplastin is incubated with human plasma. There is either no significant change or a shortening of the clotting time following incubation of other animal thromboplastins with human plasma. The practice of using heterologous thromboplastins in studies on blood coagulation may be one reason why antithromboplastin activity has not heretofore been observed in human plasma.

8. *Antithromboplastin in plasma globulin fractions.* "Platelet free" citrated plasma separated from hemophilic blood was divided into 3 portions of 10 cc. each: 1, left intact; 2, mixed with 25 mm.^{3} of concentrated human thromboplastin and incubated for 15 min.; 3, heated to 65°C. for 5 min. and cooled. To each of these portions, 90 cc. of cold distilled water was added, followed by 1.5 cc. of 1 per cent acetic acid, slowly. The cloudy precipitate was packed down by centrifugation and, after removal of the supernatant fluid, dissolved in 5 cc. of 0.85 per cent NaCl and carefully neutralized with dilute NaOH. One sample of 10 cc. of intact normal plasma, collected with the same precautions, was handled likewise. The globulin fraction isolated from hemophilic plasma exerts a clear decelerating effect on the two standard dilute thromboplastin solutions (table 7). The fraction isolated from intact normal plasma has some activity, though less than that obtained from hemophilic plasma. No activity is present in the

fractions separated from hemophilic plasma after preliminary incubation with tissue extract or after heating to 65°C. for 5 min.

One of the explanations for the slow coagulation of hemophilic blood is that its prothrombin is abnormally slow in being transformed into thrombin (2, 9). The validity of this explanation, based on the behavior of prothrombin solutions prepared by dilution and acidification of the plasma has been challenged by Howell (10). Prothrombin, isolated by his acetone precipitation method, behaves alike whether obtained from hemophilic or normal plasma. According to Howell, if dilution and acidification are used, the "plasma thromboplastin" (free thromboplastin) is brought down with the prothrombin; since hemophilic plasma has a low content of free thromboplastin, any prothrombin solution pre-

TABLE 7

Antithromboplastin activity of globulin fractions precipitated by dilution and acidification of variously treated hemophilic and normal plasmas

Figures in the body of the table represent clotting time in seconds

TEST SOLUTION OF THROMBOPL.*	SOURCE OF THE GLOBULIN SOLUTION				CONTROL†
	1. Intact hemoph. plasma	2. Hemoph. plasma + thrombopl.	3. Heated hemoph. plasma	4. Intact normal plasma	
Sol. A					
0 minute incub........	56	46	58	47	43
5 minutes incub.......	95	50	63	70	41
Sol. B					
0 minute incub........	84	74	89	87	95
5 minutes incub.......	140	82	91	110	92

* Contents of each tube: Without incubation: 0.1 cc. fresh plasma, 0.1 cc. "globulin solution," 0.1 cc. 0.025 M CaCl₂, 0.1 cc. thrombopl. sol. A (or B). With incubation: 0.1 cc. "globulin solution," 0.1 cc. thrombopl. sol. A (or B), incubation; 0.1 cc. 0.025 M CaCl₂, 0.1 cc. fresh plasma.

† Contents of mixtures as above, except that the globulin solution is replaced by 0.85 per cent NaCl.

pared from it by this method will behave differently from one prepared from normal citrated plasma, which has a higher content of free thromboplastin (10). The present experiments indicate that the difference in behavior of such prothrombin solutions must also be attributed, in part at least, to the greater content of antithromboplastin in the hemophilic plasma fractions.

COMMENT. The foregoing evidence appears to indicate that fresh plasma, *collected with special precautions*, has the property of reducing the clot accelerating action of dilute brain tissue extracts. This property is probably the manifestation of the existence in normal plasma of a substance (or group of substances) endowed with antithromboplastic activity (15). The demonstration of this property is made possible only because addition of sodium citrate to blood immobilizes the prothrombin, thereby preventing its activation by thromboplastin. While prothrombin is not in a reactive state, incubation of plasma with

the thromboplastin solution supplies an opportunity for the inactivating effect of antithromboplastin. When the plasma is recalcified, the prothrombin again becomes reactive; since, however, the potency of the thromboplastin has, meanwhile, been reduced by incubation with the plasma, the conversion of prothrombin to thrombin is correspondingly delayed. Incubation with plasma in which the antithromboplastin had already been exhausted in one way or another, would not be expected to (and does not) affect appreciably the clot accelerating action of the thromboplastin solution. Evidence is now available (4) that in the reaction between thromboplastin and prothrombin some of the thromboplastin is consumed.

When blood, shed without admixture of tissue juices, is allowed to stand "in vitro" a number of platelets (probably less than 10,000 per cmm., or the platelets at or in the immediate vicinity of the blood-glass and blood-air interfaces), swell and disintegrate, releasing a small amount of thromboplastin which is at once inactivated. This process continues until all the antithromboplastin is exhausted, and prevents the free thromboplastin from reaching a sufficiently high concentration to act at once on prothrombin. Whenever and wherever in the plasma, enough free thromboplastin is available beyond that which has been inactivated, it acts on prothrombin and transforms it into thrombin. The eventual excess of the accelerator over its antagonist in normal standing blood, assures a moderately rapid conversion of a large amount of the prothrombin. Since there is an excess of antithromboplastin in hemophilic blood, however, a correspondingly greater amount of free thromboplastin, and a longer time, will be required for this neutralization. As pointed out by several investigators (2, 11, 12) hemophilic blood or plasma can be made to clot within a normal period of time by the addition of an excessive amount of platelets or tissue extract.

Even though, at first sight, the inhibitory effect of antithromboplastin may appear small when compared with the time required for coagulation of hemophilic blood, it must be remembered that in these experiments, this inhibiting property of the plasma is being tested against larger amounts of thromboplastin than are ordinarily released, *at one time*, in blood circulating in the body or standing "in vitro." Moreover, the delay in the inception of clotting of hemophilic blood resulting from an excess of antithromboplastin must also exert, indirectly, a cumulative retarding influence on succeeding or associated changes in the blood (platelet disintegration, prothrombin transformation) and may even help to enhance the effectiveness of antithrombin. Each of these *secondary* effects, namely, slowness of platelet disintegration (6), low content of free thromboplastin in the plasma (10, 13), retarded transformation of prothrombin (9), excess of antithrombin (14), has been proposed, at one time or another, as the primary cause of the coagulation delay. The direct evidence in support of these proposals has, however, not been altogether satisfying. The abnormal stability conferred on hemophilic plasma by its excess of antithromboplastin is probably responsible for the imperfect performance of those functions (15).

SUMMARY

1. In plasma separated from normal human blood, collected with especial precautions, there is a substance (antithromboplastin) which on incubation with dilute extracts of homologous brain tissue, reduces the clot accelerating action of these extracts.

2. Antithromboplastin is exhausted during the stage preceding the inception of clotting; it has a certain degree of species specificity, is made ineffective by dilution, by heating (65°C. for 5 min.), by exposure to tissue juices, or by standing, especially in contact with blood cells.

3. By reducing the amount of available free thromboplastin released from blood or tissue cells, and thereby delaying the activation of prothrombin, antithromboplastin may play an important rôle in maintaining the fluidity of circulating blood and in postponing the inception of clotting of shed blood.

4. Hemophilic plasma has an activity 5 to 8 times greater than that of normal plasma against certain thromboplastin solutions. In standing shed hemophilic blood, more free thromboplastin and a longer time is required for the neutralization of antithromboplastin than in standing normal blood. An excess of antithromboplastin is probably the *primary* cause of the delay in the inception of clotting of hemophilic blood.

REFERENCES

(1) Nolf, P. Ergebn. d. inn. Med. u. Kinderheil. **59:** 275, 1912.
(2) Eagle, H. J. Gen. Physiol. **18:** 531, 1935.
(3) Quick, A. J. Hemorrhagic diseases and the physiology of hemostasis. C. C. Thomas, 1942.
(4) Mertz, E. T., W. H. Seegers and H. P. Smith. Proc. Soc. Exper. Biol. and Med. **42:** 604, 1939.
(5) Quick, A. J. Science **92:** 113, 1940.
(6) Minot, G. R. and R. I. Lee. Arch. Int. Med. **18:** 474, 1916.
(7) Foster, R. H. K. J. Lab. and Clin. Med. **16:** 505, 1931.
(8) Nygaard, K. K. Photoelectric study of blood coagulability. St. Louis, 1941, p. 75.
(9) Addis, T. J. Path. and Bact. **15:** 427, 1911.
(10) Howell, W. H. Bull. N. Y. Acad. Med. **15:** 3, 1939.
(11) Quick, A. J., M. Stanley-Brown and F. W. Bancroft. Am. J. Med. Sci. **190:** 501, 1935.
(12) Dam, H. and H. Venndt. Lancet **1:** 70, 1940.
(13) Patek, A. J. and F. H. L. Taylor. J. Clin. Investigation **16:** 113, 1937.
(14) Weil, P. E. Compt. Rend. Acad. Sci. **141:** 667, 1905.
(15) Tocantins, L. M. Fed. Proc. **1:** 85, 1942.

THE EFFECT OF THE SELECTION OF DATA ON THE MEAN BASAL METABOLISM AND THE VARIABILITY OF THE BASAL METABOLISM OF A LARGE SERIES OF COLLEGE WOMEN[1]

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Basal metabolism studies have been made by the workers of the North Central States Co-operative Regional Project as a part of the study of the nutritional status of college women. Early in the study it became necessary to define basal metabolism to provide a uniform method of selection of the data to be used in expressing the results. A review of the literature disclosed that several criteria were used for the expression of the mean basal metabolism.

The diversity of methods used to select data apparently has been due in part to lack of agreement in the interpretation of the concept of basal metabolism. In the minds of some workers, basal metabolism has been the lowest waking metabolism obtainable, though there is nothing implicit in the definition which makes such an interpretation necessary (Means, 1937). More recently the concept of variability in the basal metabolism has developed. Boothby et al. (1936) stated that basal metabolism, as it is measured in the laboratory, is not a lowest or fixed metabolism, but rather a random sample of the metabolism observed under certain arbitrary defined conditions. Rigid adherence to the conditions of measurement will standardize but not fix the response (Boothby et al., 1937). From this point of view the selection of the minimum value to express basal metabolism violates a fundamental principle of the theory of random sampling.

There would be little confusion if each testing laboratory would define their experimental plan and report all data collected. However, detailed comparisons have been attempted between bodies of data which ignore differences which could be due entirely to the selection of the figures employed. In fact, since most experimenters report only that part of their data which they believe pertinent, the material necessary for the evaluation of variability is lost to the reader.

Berkson and Boothby (1938) presented their studies on variability in basal metabolism. In this publication the variability present in a series of basal metabolism observations was broken down into several parts and the rôle of each defined.

Wishart (1937) has suggested that the metabolism of women, particularly those individuals not living a strictly routine life is especially subject to variation. Rogers (1939) found that a non-student group showed a more uniform basal metabolic rate than the college group and believed it probable that the more settled manner of living of the non-student group might account for this difference.

The study herein reported uses all data except those observations discarded in the laboratory for obvious technical error or non basal condition. An attempt will be made to answer two questions: What effect does the selection of figures have on the derived mean basal metabolism, and, is the character of the sample changed by selection? Standard deviations of the observations on college women by the co-operative laboratories are presented as the first contribution of this type on the variability of the basal metabolism of college women.

EXPERIMENTAL. Data are reported from the laboratories of Iowa State College, Kansas State College, the University of Minnesota, and Oklahoma Agricultural and Mechanical College. Uniform methods were used as defined in the reports of the co-operating group to study the Nutritional Status of College Women (Master Project, 1936 and Minutes of conference meetings, 1936–1940). The subjects were volunteer women students from 17 to 23 years of age. All were judged free from disease known to affect the basal metabolism. It was the intention of the investigators to study a student throughout her four years of college residence. This intention was realized within the limitations of the student's college career. Because of withdrawal from college a certain number were studied for only one year. These individuals have been identified as the "nonrepeat" group. Individuals observed for more than one year have been referred to as the "repeat" group. All academic years have been studied although first year observations only made from 1936 to 1939 are reported in this paper.[6]

The basal metabolism was measured by the Benedict-Roth closed circuit respiratory apparatus with a rubber mouth piece and a nose clip. All equipment was calibrated and checked by accepted means. The subjects came to the laboratory in the morning immediately after rising and with as little expenditure of energy as possible. They rested in bed for at least one-half hour in a room as free from external stimuli as possible prior to making the test. In general, the procedure used was to make two tests on each of two successive or nearly successive mornings, with the exception of the Oklahoma laboratory, where three tests per morning were made. The principles of the test were explained to each subject prior to the first experimental day. Careful records were kept of dates of menstruation and irregularities in the previous twenty-four hour routine. Periods of unusual campus unrest such as examination, etc., were

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[6] Because of certain group differences in the variability of the basal metabolism the segregation of students into "repeat" and "nonrepeat" series has been followed in this paper.

avoided.   All data were recorded unless at the time of the test and before calculation some error in technic was noticed or the patient was in non-basal condition. From preliminary analyses it was shown that age differences were an unimportant factor in the present analyses.

The data have been segregated by four common methods of selection: first, on the assumption that the six minute observation periods were random samples of the 24 hour basal metabolism, all data regardless of check in clinical percentage

TABLE 1

Mean metabolism and standard deviations based on different methods of selection of data. Nonrepeat data by state

SOURCE OF VARIATION	CALORIES PER SQUARE METER PER HOUR			
	All observations	All check within 5%	Lowest on each day check within 5%	First observation only
Iowa State College				
Mean....................................	34.99	35.00	34.65	35.19
Standard deviation.....................	2.82	2.55	2.59	2.84
Number of individuals.................	94	53	53	94
5% fiducial limit......................	34.70–35.27			
1% fiducial limit......................	34.61–35.36			
Kansas State College				
Mean....................................	34.45	33.75	33.23	34.94
Standard deviation.....................	3.18	2.63	2.64	3.81
Number of individuals.................	20	12	12	20
5% fiducial limit......................	33.74–35.15			
1% fiducial limit......................	33.51–35.39			
Oklahoma Agricultural and Mechanical College				
Mean....................................	33.65	34.15	33.39	34.04
Standard deviation.....................	2.21	1.95	1.91	2.42
Number of individuals.................	19	9	9	19
5% fiducial limit......................	33.24–34.05			
1% fiducial limit......................	33.10–34.19			

were averaged; second, all observations on individuals for whom both the tests within a day and the tests from day to day checked within five percent were averaged; third, the metabolism of a given individual was taken as an average of the lowest values on each of two days which checked within five percent (DuBois, 1930); fourth, only the first observation made was considered (Boothby, Berkson and Dunn, 1936).

The statistical methods and terms used are those of Snedecor (1940) or verbal recommendations of the staff of the Iowa State College Regional Statistical Laboratory.   All of the variability measures presented were obtained from data

on the same subjects rather than from the unequal groups as presented by Berkson and Boothby (1938).

RESULTS. Tables 1 and 2 give for each state the means, the standard deviations of the means and the five and one percent fiducial limits obtained by the four methods of selection of data.

TABLE 2

Mean metabolism and standard deviations based on different methods of selection of data. Repeat data by state

SOURCE OF VARIATION	CALORIES PER SQUARE METER PER HOUR			
	All observations	All check within 5%	Lowest on each day check within 5%	First observation only
Iowa State College				
Mean	35.46	35.37	34.97	35.92
Standard deviation	2.39	2.23	2.30	2.56
Number of individuals	48	35	35	48
5% fiducial limit	35.12–35.79			
1% fiducial limit	35.01–35.91			
Kansas State College				
Mean	33.61	33.89	33.54	34.11
Standard deviation	2.74	2.70	2.77	3.17
Number of individuals	22	12	12	22
5% fiducial limit	33.03–34.19			
1% fiducial limit	32.84–34.38			
University of Minnesota				
Mean	35.17	35.08	34.63	35.29
Standard deviation	2.95	2.85	2.83	2.93
Number of individuals	73	42.	42	73
5% fiducial limit	34.83–35.51			
1% fiducial limit	34.72–35.62			
Oklahoma Agricultural and Mechanical College				
Mean	34.64	34.20	33.52	35.03
Standard deviation	2.58	2.71	2.75	2.67
Number of individuals	21	11	11	21
5% fiducial limit	34.20–35.08			
1% fiducial limit	34.07–35.22			

The mean obtained by using all the data is very close to that obtained by using only those data which check within five percent. In all cases except the Oklahoma nonrepeat group, the highest mean is obtained when the first observation only was used; the lowest, by method three, from the average of the lowest observations only. Quantitatively, the differences were not great under the

conditions of this experiment in which a series of observations were made on each individual.

For example, in the Iowa nonrepeat group, 34.99 calories per square meter per hour was the mean of the sample. The one percent fiducial limits of the mean of the population from which this sample was drawn were 34.61 to 35.36 calories per square meter per hour. The mean of the observations checking within five per cent (35.00 calories per sq. m. per hr.) fell between these limits. This was true for both the repeat and nonrepeat groups of the Kansas and Minnesota data. The Oklahoma mean for tests which checked within five percent, 34.15 calories per square meter per hour, was just above the upper five per cent fiducial limit, 34.05 calories per square meter per hour but was within the one percent fiducial limit. For the repeat subjects the corresponding Oklahoma mean, 34.20 calories per square meter per hour, fell just on the lower five per cent fiducial limit. Apparently these unusual deviations of the Oklahoma data were accidents of sampling since they were not consistent. The majority of evidence tended to indicate that no bias in the mean was introduced by selection of only those data which checked within five per cent.

Selection of the lowest observation on each of two days which checked within five percent gave, in every case, a mean less than that obtained by using all observations. In all cases except the Oklahoma nonrepeat and Kansas repeat series there was less than one percent probability that the mean selected by this method was unbiased. Oklahoma nonrepeat and Kansas repeat series fall within the limits of low probability.

The means based on the first observations on each subject did not give convincing evidence of bias. They were somewhat higher in every case than the means obtained by the use of all data; however, they were not higher than the fiducial limits except in the case of the Iowa repeat group. In the latter instance, the mean obtained by this method, 35.92 calories per square meter per hour, falls just outside of the one per cent probability (35.91).

It will be noticed that the insistence upon a five per cent check very materially reduced the available information, since only from 47 to 73 per cent of the data could be used in any series. However, a more serious criticism was the introduction of bias into the variance in all cases of selection. Since a biased variance invalidates a test of significance, there is no possibility of statistical treatment of such data. If, then, statistical methods are to be used in studying basal metabolism data, the data must include all of the technically accurate observations with no selection after the results have been accepted and calculated.

In table 3 are presented, by state, analyses of variance of basal metabolism using all data obtained.

Certain practical implications may be drawn from the analyses concerning the number of observations necessary to establish the basal metabolism of a given individual. Since the variation within observations on the same day was so small, only one observation per day would seem to be necessary. Since, however, the variance between days of observation on the same individual was highly significant in the case of Iowa and Minnesota, and significant in the cases

of Kansas and Oklahoma, observations on more than one day are necessary to establish the basal metabolic rate of the individual. In the clinic one observation made by an experienced technician should be sufficient to distinguish the pathologic individual. For careful physiological work repeated observations on different days are a necessity. Since little advantage is gained in taking more

TABLE 3

Analysis of variance of basal metabolism using all observations. Nonrepeat and repeat groups by state

SOURCE OF VARIATION	CALORIES PER SQUARE METER PER HOUR			
	Nonrepeat group		Repeat group	
	D.F.	Mean square	D.F.	Mean square
Iowa State College				
Total................................	375		191	
Individuals..........................	93	24.75**	47	18.02**
Days on individual..................	94	4.611**	48	2.797**
Within day..........................	188	1.353	96	1.171
Kansas State College				
Total................................	79		87	
Individuals..........................	19	32.34**	21	23.645**
Days on individual..................	20	4.538*	22	3.5014
Within day..........................	40	2.315	44	1.7861
Oklahoma Agricultural and Mechanical College				
Total................................	114		137	
Individuals..........................	18	19.22**	20	34.62**
Days on individual..................	19	3.354*	21	1.955
Within day..........................	77	1.937	96	1.868
University of Minnesota				
Total................................			291	
Individuals..........................			72	28.63**
Days on individual..................			73	3.809**
Within day..........................			146	1.213

* Statistically significant.
** Statistically highly significant.

than one observation per day on an individual, the best proposal for the use of experimental time would be to make a single observation each morning on as many individuals as time permitted. These individuals should be checked on later days.

Table 4 presents a comparison of variability data for the college group with similar data obtained at the Mayo Clinic (Berkson and Boothby, 1938). The

calculations for the college group were made according to Snedecor (1940, chap. 17) and Winsor and Clark (1940). As expected, the inter-individual variability (s_I) was in every instance greater than the intra-individual variability (s_i) so that individuals are distinguished. Except for the relatively small Oklahoma group, the college women presented slightly but consistently higher variabilities than the Mayo Foundation group. Considering the great difference in the sample from which these data were obtained the agreement between this work and that of the Mayo Foundation figures is notable. Though the differences in the size of the standard deviations of the college groups and the Mayo Clinic group are not large enough to warrant any positive assertions, it would seem that college students did show a somewhat more variable basal metabolism which might be associated with the lack of routine in college living.

TABLE 4

Variabilities of basal metabolism of college women expressed as standard deviations in calories per square meter per hour

STANDARD DEVIATION	IOWA		KANSAS		MINNE- SOTA	OKLAHOMA		MAYO CLINIC[*]
	Repeat	Non-repeat	Repeat	Non-repeat	Repeat	Repeat	Non-repeat	
s total inter-individual..........	2.41	2.83	2.77	3.22	2.95	2.23	2.40	2.42
s_I inter-individual...............	1.95	2.24	2.24	2.64	2.49	2.63	1.77	1.81
Intra-daily intra-individual......	1.41	1.73	1.34	1.52	1.10	1.37	1.43	
Day-to-day intra-individual......	0.90	1.28	0.93	1.05	1.14	0.31	0.76	0.96
s_i intra-individual...............	1.08	1.16	1.63	1.85	1.58	1.41	1.62	1.61
Number of individuals..........	48	94	22	20	73	21	19	

[*] Berkson and Boothby (1938).

It will be noted that in each school the nonrepeat groups showed a greater variability than did the repeat group. When it is recalled that the nonrepeat group is made up for the most part of individuals who left college before they could be observed a second year, one might engage in interesting speculation, particularly since the number of tests observed for each student was the same and all tests were done in the first year in college.

SUMMARY

A study has been made of the effect which the selection of data exerts on the mean basal metabolism. Of the four methods of selection commonly employed, only one, that of averaging the lowest values on two days which checked within five per cent, gave a mean which was biased. When only the first observation on each subject was used, the mean was slightly higher than that obtained using all the data. It made little difference in mean metabolism whether all data were used or whether a selection was made involving only those data which checked within five percent. Any selection of data resulted in elimination of nearly 50 per cent of the data. However, selective sampling schemes bias the

variance. Bias of the variance invalidates the test of significance and statistical methods then cannot be applied to basal metabolism data which have been subjected to selection.

Analysis of variance indicated that in order to determine the trend of an individual's basal metabolism only one observation per day is needed, but that more than one day of observation should be used.

Standard deviations for total inter-individual, inter-individual, intra-individual, intra-daily intra-individual, and day to day intra-individual variabilities are presented for the basal metabolism of college women.

The variabilities of the repeat groups are somewhat less than the nonrepeat college groups.

REFERENCES

BERKSON, J. AND W. M. BOOTHBY. This Journal **121**: 669, 1938.

BOOTHBY, W. M., J. BERKSON AND H. L. DUNN. This Journal **116**: 468, 1936.

BOOTHBY, W. M., J. BERKSON AND W. A. PLUMMER. Ann. Int. Med. **11**: 1014, 1937.

DuBois, E. F. J. Nutrition **3**: 217, 1930.

Master Project. North Central States Co-operative Project on Nutritional Status of College Women. 1936. On file Iowa State College.

MEANS, J. H. The thyroid and its diseases. Philadelphia, J. B. Lippincott Co., 1937.

Minutes of conference meetings. North Central States Co-operative Projects. 1936–40. On file Iowa State College.

ROGERS, E. C. J. Nutrition **18**: 195, 1939.

SNEDECOR, G. W. Statistical methods. Ames, Collegiate Press, Inc., Chapter 17, 1940.

WINSOR, C. P. AND G. T. CLARKE. J. Marine Research **3**: 1, 1940.

WISHART, G. M. Quart. J. Med. **20**: 193, 1927.

THE EFFECT OF MENARCHE ON BASAL PHYSIOLOGICAL FUNCTIONS IN GIRLS[1]

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Previous investigators have found that physiological precocity, as indicated by early menarche in girls, is usually associated with precocity of growth in body size. Even at the age of 8 or 9 years, girls who begin menstruation at the age of 10 or 11 years, are taller and heavier than girls who do not begin to menstruate till they are 14 or 15 years of age (8, 10, 11). However, few studies of the physiological differences between early and late maturing girls have been made. The following study was planned to throw light on this question and to determine the effects of menarche on basal physiological functions in normal girls

EXPERIMENTAL. Subjects for this study (50 girls) were chosen from the public schools of Oakland, California, solely on the basis of the willingness of their parents to co-operate in a long-term study and the probability of their permanent residence in the community. No physical or physiological characteristic of the child was used as a criterion of selection. The children were first tested when they were between the ages of 11 and 12 years (mean age 11.87 years; S.D. 0.5 year) and were re-tested at six month intervals over a six year period. The following test procedure was followed on each of two successive mornings: The children were brought to the laboratory by automobile before breakfast. Upon arrival, each child lay down on a cot for a 20-minute rest period before measurements were recorded. Systolic blood pressure was read by the auscultatory method at the first sound and diastolic pressures at the fourth sound. The mean of three determinations made at 8-minute intervals was used for tabulation. Pulse rates were counted over half-minute intervals immediate following each blood pressure determination. The average of three observations was used in the tabulations. Basal oxygen consumption was measured over three 8-minute periods by the Tissot open circuit method (3). Siebe-Gorman half-masks were used for collecting the expired air, and expired air was separated from inspired air with Bailey mercury valves (1). The basal respiratory volumes were computed, reduced to standard conditions of 0°C. and 760 mm. Hg. The average values for tests made on the two successive days

were calculated for each subject. These averages were used in the present analysis.

Each month a report was obtained from each girl of the day on which the menstrual flow began and the day on which it ended. In this way accurate dates of first menstruation were obtained. The age of menarche used in this study was the age at the first reported flow, whether regularly maintained thereafter or not. Many irregular periods were reported during the first several months of menstruation in this group of girls (2).

RESULTS. For each girl the time (or age) at which the physiological tests were made was expressed as months before or after menarche, instead of as chronological age. Mean values of blood pressure, pulse rate, basal respiratory volume and basal oxygen consumption were computed for each six month interval before or after menarche.

The results of this analysis are shown in curves plotted as figures 1, 2, 3 and 4. If at menarche there were a sudden change in physiological conditions within the body which produced changes in blood pressure, for instance, curves plotted in the manner outlined above would show a sudden change in slope or direction of trend at the time of menarche. Examination of the curves of figure 1 shows that rising systolic blood pressure in girls occurs prior to menarche and that with the beginning of menstruation, or shortly thereafter, the systolic blood pressure reaches a level beyond which no systematic change occurs. No systematic changes in diastolic blood pressure were observed. The pulse pressure changes (fig. 2) are primarily a reflection of the changes in systolic pressure that occur at menarche.

Figure 2 shows that basal pulse rate rises in girls over the pre-menarcheal period. In the present group of girls the pulse rate began to fall, on the average, 6 to 12 months prior to the beginning of menstruation. The basal pulse rate of girls continues to decrease throughout the remainder of the adolescent period.

At menarche there is a sudden cessation in the increase in basal respiratory volume which has been proceeding throughout the pre-pubertal period (fig. 3). Since total respiratory volume is influenced by body size, basal respiratory volumes were calculated per unit of surface area for each girl. (Surface areas were computed from height and weight by the DuBois (5) formula.) With this correction for the effect of body size, values for basal respiratory volume begin to decrease prior to menarche and decrease more rapidly in the two years following menarche.

Figure 4 shows that prior to menarche the basal oxygen consumption in girls has attained more or less stable values at a relatively high level of consumption. With the beginning of menstruation (or even 6 to 12 mos. before), there is a sudden rapid fall in basal oxygen consumption. Thus the decrease in basal metabolism which characterizes the adolescent period takes place rather rapidly over the two or three years following menarche.

DISCUSSION. The results of this study do not lend support to the view that the beginning of menstruation in girls produces any sudden alteration in physiological conditions which are reflected in pulse rate, blood pressure or basal

metabolism. Although there may be a quickening of the rate of change in oxygen consumption and pulse rate, for instance, after menarche, the curves show that in many individual girls the decrement began 6 to 12 months before
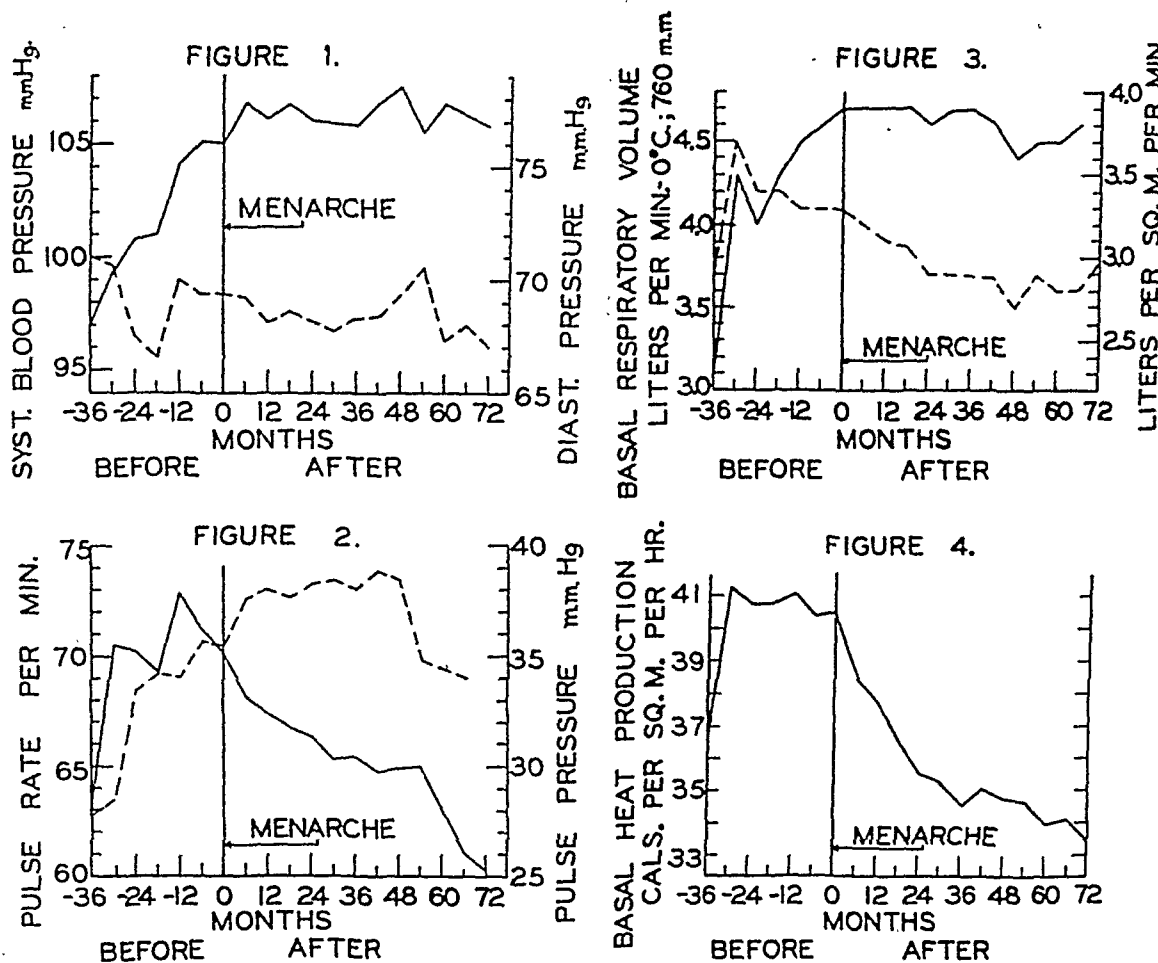
Fig. 1. Effect of menarche on blood pressure in girls. Average values calculated for 6-month intervals before and after menarche for 50 girls. Zero time is taken for each girl at the age when she first menstruated.

————— systolic blood pressure; ----------- diastolic blood pressure.

Fig. 2. Effect of menarche on pulse rate and pulse pressure in girls. See legend of figure 1 for complete description.

————— pulse rate; ----------- pulse pressure.

Fig. 3. Effect of menarche on basal respiratory volume in girls. See legend of figure 1 for complete description.

————— liters per minute; ----------- liters per square meter per minute.

Fig. 4. Effect of menarche on basal heat production in girls. See legend of figure 1 for complete description.

————— basal heat production, calories per square meter per hour.

menstruation occurred. Thus it is safer to regard this period of rapid fall in metabolism as another aspect of maturity which ordinarily occurs about the same time as the first menstruation, rather than the result of endocrine changes associated with menstruation. The changes observed in all the physiological

functions measured are in the direction of adult values and take place at a more rapid rate after menarche than before. Selection of groups of adolescents on the basis of a physiological criterion, such as age at menarche, results in greater homogeneity of the group with respect to other physiological criteria, such as pulse rate, blood pressure or oxygen consumption, than does selection on the basis of chronological age. The periods of rapid change which occur with menarche can be observed in individual growth curves but may be entirely obscured when average curves, based on chronological age, are examined (9). This obscurity is reflected in the disagreement found in the literature on basal metabolism as to the existence of a pre-pubertal rise in basal oxygen consumption (4, 6, 7, 12, 13). The observations of the present study do not begin at an early enough chronological age to answer the question with assurance for girls. However, in many of the girls studied, particularly those who menstruated later, a pre-pubertal rise in metabolism was observed. A rapid fall in oxygen consumption was observed by Johnson (6) in 6 tubercular girls whom he studied continuously over 300 days. Associated with the fall in basal oxygen consumption there was a marked drop in Ca and N retention which continued for 12 to 30 days after menarche.

SUMMARY. Determinations of pulse rate, blood pressure, respiratory volume and heat production under basal conditions were made on each of 50 normal girls at intervals of 6 months between the ages of 11.5 and 17.5 years. All determinations were made under basal conditions on two successive mornings at each six month testing. The Tissot open circuit method was used for estimating basal oxygen consumption during three 8-minute periods on each of the two mornings. Accurate menstrual histories were kept for each subject.

Analysis of curves relating the measured functions to age for each girl shows three marked changes associated with the beginning of menstruation; 1, a leveling off of a pre-menarcheal rise in systolic blood pressure; 2, a decrease in pulse rate, and 3, a precipitous fall in basal heat production per unit of surface area. None of these phenomena are observable in average growth curves based on chronological age because of variations in the age at which onset of menstruation occurs among normal girls.

CONCLUSIONS

1. With the beginning of menstruation there is a rapid decrease in basal oxygen consumption in girls.

2. This period of rapid fall in oxygen consumption is more closely associated with menarche than with any particular chronological age.

3. With the attainment of menarche, other physiological functions, such as blood pressure and pulse rate, rapidly approach mature levels and become stabilized.

REFERENCES

(1) BAILEY, C. V. Proc. Soc. Exper. Biol. and Med. 24: 184, 1926.
(2) BAYER, L. M. J. Pediat. 17: 345, 1940.

(3) Boothby, W. M. and I. Sandiford. Laboratory manual of the technique of basal metabolic rate determinations. Philadelphia, W. B. Saunders Co., 1920.

(4) Bruen, C. J. Nutrition 6: 383, 1933.

(5) Du Bois, D. and E. G. Du Bois. Arch. Int. Med. 17: 863, 1916.

(6) Johnson, J. A. Am. J. Dis. Child. 59: 287, 1940.

(7) Lewis, C. A. Am. J. Dis. Child. 51: 1014, 1936.

(8) Richey, H. G. The relation of accelerated, normal and retarded puberty to the height and weight of school children. Monogr. Soc. for Res. in Child Develop. 2: 1, 1937.

(9) Shock, N. W. Am. J. Dis. Child. 64: 19, 1942.

(10) Shuttleworth, F. K. Sexual maturation and the physical growth of girls age six to nineteen. Monogr. Soc. for Res. in Child Develop. 2: 1937.

(11) Stone, C. P. and R. G. Barker. Human Biol. 9: 1, 1937.

(12) Talbot, F. G., E. B. Wilson and J. Worcester. Am. J. Dis. Child. 53: 273, 1937.

(13) Topper, A. and H. Mulier. Am. J. Dis. Child. 43: 327, 1932.

THE MECHANISM OF EXPERIMENTAL RENAL HYPERTENSION IN THE RAT: THE RELATIVE SIGNIFICANCE OF PRESSOR AND ANTI-PRESSOR FACTORS[1]

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The present paper is based on observations made on more than a thousand adult rats in which hypertension had been induced by various operative procedures on the kidneys. The results obtained are in certain respects contradictory to many of the generally accepted concepts concerning the mechanism and treatment of hypertension. They are presented here for their possible value in further elucidating the general problem of experimental and clinical hypertension.

METHODS. Rats have been used exclusively in the present study. Most of the animals were of a piebald strain reared in the laboratory; the remainder, of the Wistar strain, obtained from dealers. The blood pressures were determined by the procedure previously described (1). Hypertension was induced by the application of cloth to the kidney (2, 3).

All operative procedures were carried out under ether and rarely required more than a few minutes for their completion. Attempts were made to minimize trauma, which causes marked drops in the blood pressure of hypertensive animals.

EXPERIMENTAL OBSERVATIONS. *The effect of unilateral nephrectomy.* The failure of unilateral nephrectomy to induce hypertension consistently is well known. However, occasionally hypertension does develop following the removal of one kidney from an apparently normal animal. This is illustrated by the following experiment: The blood pressures of a number of normal rats were determined daily until reasonably constant values were obtained for a period of at least 10 days. The right kidney was then removed under ether anesthesia and the daily blood pressure readings continued. During the week following the operation the general average of the systolic blood pressure of most of the animals was elevated, although only temporarily and to a degree so slight as to be of questionable significance. The subsequent course of the blood pressure varied but, in general, followed one of three courses. In about half of the animals the slight elevation observed post-operatively gradually disappeared and the original pre-operative level was resumed within 10 to 20 days. In most of the other animals, however, the post-operative level remained at a slightly elevated level (5 to 15 mm.). In an occasional animal, on the other hand, the blood

pressure remained elevated following unilateral nephrectomy and assumed a definitely hypertensive level of 150 mm. or more which was sustained thereafter.

Although unilateral nephrectomy induces an elevation in blood pressure to a truly hypertensive level in only an occasional rat, the general average pressure in unilaterally nephrectomized animals is definitely higher than in normal animals. For example, in a group of 20 rats, the average systolic blood pressure on repeated determinations was 120, with individual variations of 113 to 126. The average blood pressure level of the same animals during the week following nephrectomy was only slightly altered from the pre-operative level. However, thereafter, the blood pressure as determined at intervals on 20 occasions during the next six months varied between 124 and 138 mm. with a general average of 128 mm. There was thus a definite—although slight—elevation in blood pressure induced by unilateral nephrectomy.

The effect of other unilateral operations on the kidney. The application of silk to one kidney, partial removal of a single kidney and other operative procedures such as applying a partial ligature to the renal artery, all result in effects similar to those described in the preceding section. In many animals these procedures result in a minimal and temporary elevation of blood pressure with a resumption of the normal pre-operative level in the course of two to three weeks. In some otherwise apparently normal animals, operative interference with one kidney (as in the case of unilateral nephrectomy) results in a permanently elevated blood pressure. In others a slight elevation only is induced.

If experimental renal hypertension be due to a liberation of renin, or of an activated renin (angiotonin or hypertensin), it would be expected that the unilateral application of cloth or collodion, for example, or other operative procedure on the kidney, should result in a greater elevation of blood pressure than simple removal of a kidney. In fact, nephrectomy alone could not induce hypertension except by secondary changes which such removal might induce in the remaining kidney. To test this point a group of 20 rats were unilaterally nephrectomized, while in an equal number a collodion capsule was applied to one kidney. The results of this study were as follows:

The average pre-operative blood pressure of the 20 rats was 120, which was identical with that of the group which were unilaterally nephrectomized. The average blood pressures on 15 subsequent determinations from one to six months following the application of collodion to one kidney was 130 mm. as compared to 128 mm. in a similar group of animals which had been unilaterally nephrectomized. This difference of 2 mm. is statistically insignificant in view of the large variations observed in the pressures of the individual animals. However, one point of difference between the two groups may be significant; whereas, only two of the 20 nephrectomized animals died during the period of observation, nine of the group in which collodion was applied to the kidney succumbed between one and six months following operation. This increased mortality may have been due to secondary infection with abscess formation which is not uncommon in animals subjected to the "collodion" operation.

The effect of removing a kidney from rats in which hypertension has been induced

by silk applied to a single kidney or by other operation. The view is widely accepted that experimental interference with the kidneys results in hypertension due to the liberation by the kidney of a pressor substance. If this be true one would anticipate a drop in blood pressure to the pre-operative level following the removal of a kidney to which silk had been applied, or which had been otherwise manipulated to give an elevation of pressure.

The experiments cited in table 1 were carried out to test the above hypothesis. It is evident from these experiments that removal of the presumably injured kidney did not cause the blood pressure to return to the normal level but actually resulted in no change in the blood pressure or in a slight further elevation of the blood pressure.

The effects of bilateral nephrectomy in hypertensive rats. It has long been known that bilateral nephrectomy does not result in the development of hypertension. The explanation for this phenomenon has been that with the removal of all renal

TABLE 1

The effect of removing a kidney to which silk or collodion had been applied with the subsequent development of hypertension on the systolic blood pressure (expressed in millimeters of mercury)

RAT NO.	ORIGINAL PREOPERATIVE BLOOD PRESSURE	AVERAGE BLOOD PRESSURE LEVEL 2 MONTHS AFTER APPLICATION OF SILK TO ONE KIDNEY	POST-OPERATIVE LEVEL			
			1 week	2 weeks	3 weeks	4 weeks
1	120	140	150	155	150	150
2	130	155	150	155	160	160
3	125	150	145	145	155	150
4	130	160	155	160	160	170
5	130	150	160	165	170	170
6	120	145	150	150	150	155
7	115	170	175	175	170	170
8	120	150	150	165	160	160

tissue there remains no possibility for the production of the hypothetical pressor substance responsible for hypertension. To determine if the absence of all renal tissue was compatible with hypertension, one kidney of the rat was removed, and in those cases in which hypertension did not follow, the remaining one was either partially removed or surrounded with silk. When the blood pressure level had reached a constant level for a period of at least 10 days the remaining kidney was freed of adhesions under ether anesthesia and the incisions closed with single ligatures. The purpose of this preliminary operation was to avoid as much trauma as possible when this remaining kidney was removed and thus have the animal in good post-operative state. Otherwise, the effect of shock and uremia would in themselves tend to lower the blood pressure.

The results of these experiments indicated that following the removal of the remaining kidney, the blood pressure did not drop precipitously but tended to gradually assume the shock levels observed pre-mortem in all hypertensive animals, as shown in the experiments cited in table 2.

Comparison of the effects of bilateral nephrectomy, ligation of the ureters and ischemia of the kidneys. Further evidence of the relative effects of deprivation of renal tissue, and the presence of abnormal renal tissue on the blood pressure was obtained by comparing the results of nephrectomy, ligation of the ureter and ischemia of the kidney in animals from which one kidney had been previously removed. This preliminary operation was performed in order to reduce the shock attendant on the bilateral operation. One month after this nephrectomy the blood pressure of the animals was determined daily until relatively constant values were obtained. The animals were then divided into three equal groups. The remaining kidney of one group was removed; the ureter in the second group was ligated and the renal artery of the third group was clamped for thirty minutes. The blood pressures were then determined each morning and afternoon subsequently.

The results of the above described operations indicated that in the rat with complete absence of renal tissue the blood pressure rises in a manner and to an extent equal to that observed in the presence of one kidney the ureter of which

TABLE 2

The blood pressure of hypertensive animals following the removal of their remaining kidney

TYPE OF OPERATION PERFORMED	ORIGINAL BLOOD PRESSURE LEVEL	AVERAGE SYSTOLIC BLOOD PRESSURE LEVELS IN MILLIMETERS OF MERCURY				
		Before final nephrectomy	Following nephrectomy			
			Day 2	Day 3	Day 4	Day 5
Application of silk......	110	170	170	180	160	100
Application of silk......	115	180	170	180	160	90
Partial nephrectomy....	100	190	180	160	100	
Partial nephrectomy....	95	200	170	170	120	

had been ligated. Were the rise in blood pressure attributable to the production by the kidney of a pressor substance, one would have anticipated a greater rise in blood pressure in the group of animals in which renal tissue was present.

Ischemia for 30 minutes also resulted in a temporary rise in pressure but this rise was less and of shorter duration than of that observed in the nephrectomized animals or in those in which the ureter was ligated. It is possible that this temporary rise may be due to the liberation from the ischemic kidney of a pressor substance of the nature of angiotonin or hypertensin, but one could equally well explain the observed result as being due to a temporary cessation of renal activity, which in the case of the ischemic kidney is restored after some hours but which is permanent in the case of the nephrectomized animals or those in which the ureter is ligated.

DISCUSSION. Our results are similar in principle but differ somewhat in detail from those recently reported by Friedman, Jarman and Klemperer (4), who also found a failure of the blood pressure to return to normal following re-

moval of the affected kidney. However, these observers reported that unilateral application of cellophane resulted almost uniformly in a steadily increasing blood pressure. Furthermore, pre-operative blood pressure levels of their rats were definitely higher (about 20 mm.) than those which we have observed consistently in our own colony. Whether these differences are to be ascribed to variations in technique, in diet, or in training of the animals is uncertain.

The pathological findings observed by Friedman, Jarman and Klemperer were also different from those which we commonly find following the application of silk unilaterally. If the silk be applied loosely without contraction of the pedicle no superficially observable abnormalities such as were noted by Friedman, Jarman and Klemperer ensue. It is possible, therefore, that the more pronounced tissue destruction induced by the application of cellophane may have caused the liberation of pressor substances not formed when the looser silk is applied. Against this view is the fact that, in our animals, application of collodion unilaterally, which also induces marked destruction of renal cortex, or the ligature of one renal artery also produced only temporary elevation of blood pressure in the majority of animals.

The current views as to the genesis of renal hypertension are 1, that it is due to the production of an abnormal pressor substance by the kidney; 2, that it results from the absence of a normal renal constituent, and 3, that it is due to an imbalance in the ratio of a pressor and depressor substance, both of which are elaborated by the kidney. According to the first view, hypertension is due to renal injury or ischemia resulting in the production of a noxious pressor substance. The second view assumes that the normal kidney has an internal secretory activity which when interfered with results in the hypertension. The last view assumes that renal hypertension is dependent on a disturbed balance between pressor and depressor constituents.

The results of the present study conform most closely to the second of the above-mentioned hypotheses. According to this view the increase in blood pressure which sometimes follows unilateral nephrectomy is due to an incipient deficiency induced by removing functional tissue.

The fact that removal of a kidney to which silk has been applied with the resulting development of hypertension did not cause a return of the blood pressure to normal and the behavior of parabiotic rats (5) also speaks in favor of the "deficiency" theory. Were hypertension due to the production of an abnormal pressor substance or to an imbalance between pressor and depressor or antipressor bodies one would anticipate that removal of such a kidney would cause decline in blood pressure. Such was not the case in these experiments.

It may be argued, of course, that the occurrence of hypertension following removal of one kidney and its failure to be alleviated by removal of a kidney to which silk had been applied or in which hypertension had been otherwise induced, is due to a pressor mechanism set in action by irreversible changes in the second kidney. However, the fact that removal of this second kidney does not cause a reduction in blood pressure as well as the observation that bilateral

nephrectomy causes a measurable (although slight) increase in blood pressure makes this possibility unlikely.

It should be emphasized that the work reported in this communication has been done on rats and that the discussion applies only to experimental renal hypertension as observed in these animals. There is considerable evidence derived from the work of other investigators, that pressor mechanisms may be concerned in the genesis of renal hypertension in the dog. On the basis of the available evidence it does not seem justifiable to conclude either that such mechanisms are active in the rat or that they are absent in the dog. In both species it would appear that deficiency of an anti-pressor mechanism is an important factor in the genesis of experimental renal hypertension.

SUMMARY

Unilateral nephrectomy or the application of cloth or collodion to one kidney caused hypertension in a small percentage of normal rats. The subsequent removal from such animals of the kidney to which cloth or collodion had been applied did not result in a significant decline of the elevated blood pressure. Total nephrectomy in animals surviving sufficiently long tended to cause an elevation of blood pressure. The bearing of these experiments on the theories of the pathogenesis of experimental renal hypertension is discussed.

REFERENCES

(1) WILLIAMS, J. R., JR., T. R. HARRISON AND A. GROLLMAN. J. Clin. Investigation 18: 373, 1939.

(2) PAGE, I. H. J. A. M. A. 113: 2046, 1939.

(3) GROLLMAN, A. AND J. R. WILLIAMS, JR. Am. J. Med. Sci. 204: 73, 1942.

(4) FRIEDMAN, B., J. JARMAN AND P. KLEMPERER. Am. J. Med. Sci. 202: 20, 1941.

(5) GROLLMAN, A. AND C. RULE. This Journal 138: 587, 1943.

CHANGES IN SERUM PHOSPHATE AND CALCIUM AND THEIR RELATION TO MANIFESTATIONS OF TRAUMATIC SHOCK[1]

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During the course of experiments (1) involving the production of shock in dogs by the tourniquet method, the phosphates of the serum were observed to rise as the manifestations of the condition developed. This is reported (2) to occur, also, when shock is induced by repeated bleeding. The study that follows was designed to determine the significance of the increase in serum phosphate.

Effects on infusion of phosphates and their influence on calcium were investigated. The relation of calcium to capillary permeability led to the study of citrates, known to have specific influence on the ionization of calcium. Atropine also was used to aid in the interpretation of the part played by the autonomic nervous system.

Changes in pressure and constituents of the blood and in the electrocardiogram are reported following: I. The release of arterial occluding tourniquets. II. The infusion of phosphates. III. The infusion of citrates. IV. The infusion of citrates in atropinized dogs.

Group I. Changes in pressure and constituents of the blood and in the electrocardiogram following the release of arterial occluding tourniquets on the hind legs of dogs. This group includes 5 animals. They were anesthetized with nembutal, 30 mgm. per kgm., followed by 5–10 mgm. per kgm. when necessary; the tourniquets, applied for 5 hrs., were then released and the femoral artery cannulated for blood pressure determinations. In table 1 are presented changes found in blood pressure, hematocrit, plasma protein, serum calcium, serum phosphate and relative electrical ventricular systole.

With release of the tourniquets, the blood pressure falls and requires $1\frac{1}{2}$ to $2\frac{1}{2}$ hrs. to reach shock level. Other changes include hemoconcentration, loss in plasma protein and marked increase in serum phosphate, accompanied by slight if any change in the total serum calcium. The altered serum phosphate may have a minor tendency to correct itself in the later stages.

Carter and Andrus (3) were the first to describe the prolongation of the electrical systole (Q-T interval) in hypocalcemia.[2] It has been shown (4) that the Q-T interval is in constant relation to the pulse period or the R-R interval. The formula expressing this relationship was found to be $Q\text{-}T = K\sqrt{R\text{-}R}$. This K factor was definitely increased in the two animals for whom it was determined.

[2] Acknowledgment is gratefully made to Dr. E. Cowles Andrus for bringing this to our attention and to Dr. A. Geiger for valuable assistance in the interpretation of the electrocardiograms.

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Group II. Changes in pressure and constituents of the blood and in the electrocardiogram following phosphate infusion. Five animals are included in this group. As soon as the nembutal anesthesia was adequate and preparation had been completed for recording the blood pressure in the femoral artery, infusion was begun. Sodium pyrophosphate was used in the majority but, as is indicated

TABLE 1

Changes in blood pressure, blood constituents and electrocardiogram following the release of arterial occluding tourniquets

TIME IN RELATION TO TOURNIQUET PROCEDURE	B.P.	HEMA-TOCRIT	PLASMA PROTEIN	SERUM Ca	SERUM P	$K = \dfrac{Q - T}{\sqrt{RR}}$ (EKG)	REMARKS
	mm. Hg	per cent	gm. per cent	mgm. per cent	mgm. per cent		
Before application........		38.3		9.1	2.3	0.333	Dog 1: sacrificed 3
Before release............	180	40.2		10.1	3.5	0.332	hrs. after release
10 minutes after release...	148	47.8		10.1	6.14	0.378	of tourniquet
30 minutes after release...	110	48.0		10.0	5.8	0.360	
155 minutes after release..	96	45.0		9.0	5.0	0.372	
Before application........		42.0	5.22	10.4	3.4	0.324	Dog 2: died 13 hrs.
Before release............	170	40.0	5.43	11.0	3.28	0.336	after release of
15 minutes after release...	126	53.5	5.64	12.1	5.94	0.344	tourniquet
55 minutes after release...	100	55.8	4.90	9.6	5.90	0.320	
160 minutes after release..	82	56.8	4.73	8.4	7.94	0.353	
Before application........		45.8	5.44		3.34		Dog 3: sacrificed 2½
Before release............	205	51.8	5.34		5.04		hrs. after release
10 minutes after release...	146	58.9	5.09		6.60		of tourniquet
40 minutes after release...	164	68.3	5.80		7.60		
80 minutes after release...	128	65.0	5.28		7.28		
140 minutes after release..	80	58.9			6.56		
Before application........				9.4	2.28		Dog 4: sacrificed 1½
Before release............	170			9.5	3.54		hrs. after release
12 minutes after release...	140			9.7	5.1		of tourniquet
41 minutes after release...	138			9.7	6.14		
90 minutes after release...	70			9.6	6.34		
Before application........		43.7			2.5		Dog 5: sacrificed 2½
Before release............	150	40.9			2.7		hrs. after release
10 minutes after release...	126	44.5			6.0		of tourniquet
50 minutes after release...	94	52.4			6.0		
120 minutes after release..	90	55.2			6.1		

for animal III (table 2), equivalent amounts of sodium phosphate acted similarly. The infusion solution contained 3.3 per cent sodium pyrophosphate in distilled water, adjusted to pH 7.4 with hydrochloric acid. It was introduced at the rate of 1 to 4 cc. per minute, the rate being determined by the general behavior of the animal and particularly by the blood pressure and respiration.
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Analysis of table 2 shows a progressive decline of the blood pressure and a marked increase in relative packed red cell volume. Furthermore, the calcium of the serum diminished as the phosphate content increased. This is in agreement with findings of Binger (5). The relative prolongation of the electrical ventricular systole is great as indicated by K values.

TABLE 2

Changes in blood constituents and electrocardiogram accompanying the infusion of sodium pyrophosphate in anesthetized dogs

TIME AFTER START OF INFUSION	B.P.	HEMATO-CRIT	PLASMA PROTEIN	SERUM Ca	SERUM P	EKG $K = \dfrac{Q-T}{\sqrt{RR}}$	TOTAL AMOUNT INFUSED
minutes	*mm. Hg*	*per cent*	*gm. per cent*	*mgm. per cent*	*mgm. per cent*		
0	155					0.359	Dog 1: 4.3 grams sod. Pyroph. [dog died 46 minutes after start of infusion]
22	50					0.438	
37	33					0.417	
0	142	31.3	4.73				Dog 2: 3.63 grams sod. Pyroph. [dog died 107 minutes after start of infusion]
25	98	37.9	4.72				
60	48	45.7	4.94				
100		53.0	5.22				
0		34.8	4.96		3.34		Dog 3: 12.36 grams sod. Phosphate [dog died during following night]
10		39.3	5.06		5.9		
30		40.0	5.27		5.1		
120		54.5	4.32		5.0		
180		44.5					
0	144	38.4		9.6	4.34		Dog 4: 3.3 grams sod. Pyroph. [dog died 40 minutes after start of infusion]
25	80	46.1		3.9	7.16		
0	130	36.0		9.0	2.9	0.326	Dog 5: 3.3 grams sod. Pyroph. [dog died 2 hrs. after termination of infusion]
37	100	39.2		4.4	5.9		
75	70	39.3		3.9	6.6	0.379	
135	67	42.0		3.5	6.7	0.367	
200	42	46.5		4.9	4.5	0.330	

It should be recorded here that tetanic muscular activity made its appearance in all of the animals of this group during the second quarter of the infusion period and became increasingly marked as the serum calcium declined.

Compared with alterations in the same factors after release of tourniquets (group I) the outstanding features in this series are loss of serum calcium, increase in K values and outspoken tetanic muscular activity.

Group III. Changes in pressure and constituents of the blood and in the electrocardiogram following citrate infusion. Five animals are included in this series. The procedure used in the preceding group was followed in detail. The only

variant was substitution of sodium citrate for phosphate. The citrate was a 2 per cent solution in distilled water, adjusted to pH 7.4.

The first striking difference between the effects of citrate and phosphate infusion is the blood pressure response. The gradual progressive and marked fall associated with the phosphate contrasts with only slight change when citrate is used. The pressure falls abruptly only during the last few minutes of the animal's life.

With citrate there is only a moderate increase in packed cell volume, nevertheless the loss in plasma protein is considerable and exceeds what occurs with

TABLE 3

Changes in blood constituents and electrocardiogram accompanying the infusion of sodium citrate in anesthetized dogs

TIME AFTER START OF INFUSION	B.P.	HEMATO-CRIT	PLASMA PROTEIN	SERUM Ca	SERUM P	$K = \dfrac{Q-T}{\sqrt{RR}}$	TOTAL AMOUNTS INFUSED AND REMARKS
minutes	*mm. Hg*	*per cent*	*gm. per cent*	*mgm. per cent*	*mgm. per cent*		
0	115	39.2	4.29	9.9	4.4	0.341	Dog 1: 3.4 grams citrate
18	125	40.3	4.20	10.1	2.0	0.465	[dog died]
56	116	47.1	3.41	10.3	1.4	0.471	
0	90	47.9	5.82	11.3	5.1	0.289	Dog 2: 5.4 grams citrate
16		47.1	5.12	13.4	4.75	0.381	[dog died]
36	75	48.5	5.13	13.6	4.52	0.421	
60		44.2	4.60	13.8	3.52		
80	87	46.1	3.39		3.00	0.362	
0		26.0	5.16	10.8	2.96	0.339	Dog 3: 3.6 grams citrate
20		25.9	4.59	11.5	4.00	0.476	[dog died]
48		30.5	4.53	11.5	3.68	0.366	
0	98	36.5	6.13				Dog 4: 4.8 grams citrate
38	105	35	5.42				[dog died]
0	160	38.7	6.26				Dog 5: 5.6 grams citrate
40	126	39.8	5.47				[dog died]
68	108	44.6	4.64				

phosphate though this is associated with much greater increase in relative packed red cell volume. The serum calcium actually tends to rise as opposed to its fall with phosphate but the tetanic state associated with the two substances cannot be differentiated and the relative electrical ventricular systole is prolonged with citrate as it is with phosphate even though the total serum calcium certainly is not reduced with the former (6).

These facts question the accepted theory concerning the relation of the relative electrical ventricular systole, as indicated by the K factor, to the total calcium of the serum. They suggest that the K factor may be related to a particular calcium fraction. That this may be ionized calcium is indicated

by the fact that the tetanic, muscular contractions manifested equally after citrate or phosphate infusion are relieved promptly in both circumstances by injection of small amounts of ionized calcium salts.

Group IV. Changes in pressure and constituents of the blood and in the electro-cardiogram following citrate infusion in atropinized dogs. Five animals comprise this series. The procedures were identical to those for group III with the single exception that the animals were given 0.3–0.5 mgm. per kgm. of atropine sulphate intravenously five minutes before the beginning of the infusion of citrate.

The outstanding difference between the atropinized and non-atropinized dog is the amount of citrate tolerated. With the latter, death invariably terminated the experiment in about 1 hour when 3.4 to 5.4 grams of citrate had been infused. After atropine more than twice this amount of citrate was infused, with tetanic muscular activity as the only untoward symptom.

Naturally more than twice the time for the larger quantity of citrate was required as all of the infusions were made at approximately the same rate. During this longer period for the atropinized animals the blood pressure was sustained at the pre-injection level or even higher and all five dogs were in fair condition when the infusion was discontinued.

Dog 2 received 14 grams of citrate and died after an hour; dog 4 lived 4 hrs.; the other three (1, 3 and 5) recovered completely from the experimental procedures. The tetanic muscular contractions could be stopped with calcium chloride, and the alterations in the K values and changes in the blood constituents including serum calcium and phosphate, conform to those of group III. There is some hemodilution after atropine itself, but only slight variation in relative packed red cell volume occurs during the remainder of the procedure. The plasma protein loss, an outstanding feature with the non-atropinized dog, is materially reduced, but is still noteworthy. It bears no constant relation to fluid loss from plasma. All of these factors are included in the table 4.

DISCUSSION. The increase in serum phosphate following the release of tourniquets applied for 5 hrs. to the hind legs of dogs raises the question concerning its source. The fact that there is also an elevation of serum potassium (7) is supporting evidence that these electrolytes come from tissue cells distal to the tourniquets that are damaged during the period of ligation.

When cells are so altered that they lose salts like potassium and phosphate other cellular functions also may be modified with the result that altered metabolic products are liberated and, with release of the tourniquets, absorbed in sufficient quantity to participate in the production of shock. This hypothesis is by no means new but adequate support has not been forthcoming (8).

The augmented serum phosphate may be an expression both of a major discharge of this electrolyte into the circulation and a coincident depression of the ability of the kidneys to eliminate it. The latter condition is associated with the slowed circulation. An additional reason for the reduced excretion may be the nembutal anesthesia.

The rise in serum phosphate from 2 mgm. per cent to as much as 7.6 mgm.

per cent after the release of the tourniquets is only relatively important, if the following premises are correct. The effects of phosphate are associated primarily with its influence on calcium. As has been shown, the total serum calcium is practically unaffected and the ionized calcium, as far as this may be evaluated

TABLE 4

Changes in blood constituents and electrocardiogram accompanying the infusion of sodium citrate in anesthetized atropinized dogs

TIME AFTER START OF INFUSION	B.P.	HEMATO-CRIT	PLASMA PROTEIN	SERUM Ca	SERUM P	EKG $K = \dfrac{Q-T}{\sqrt{RR}}$	TOTAL AMOUNTS INFUSED
minutes	mm. Hg	per cent	gm. per cent	mgm. per cent	mgm. per cent		
0		28.2	5.51	10.2	3.2	0.283	Dog 1: 10 grams citrate
30		28.9	4.59	11.2	2.4	0.359	[dog survived]
60		37.6	4.81	10.1	2.0	0.359	
90		38.4	4.50	9.3	1.5	0.347	
150		40.1	5.09	9.6	1.4	0.347	
0*	66	36.8	5.25	11.0		0.344	Dog 2: 14 grams citrate
0	66	28.1	4.73	10.5		0.348	[dog died 1 hr. later]
54	60	28.9	4.29	12.0		0.389	
102	80	30.1	4.04	14.0		0.422	
147	80	32.0	4.01	12.5		0.398	
0*	142	41.9	5.13	10.4	4.0		Dog 3: 10.5 grams citrate
0	142	33.8	5.05	10.4	4.2		[dog survived]
42	135	33.0		13.1	4.2		
85	108	40.0	4.32	13.1	4.4		
140	140	40.0	3.85	11.4	4.4		
0*		43.8	5.16	10.7	3.3		Dog 4: 7 grams citrate [dog
0		33.6	4.97	10.8	3.6		died 4 hrs. later]
43		41.0	5.03	10.8	2.7		
91		43.8	4.85	10.1	1.9		
138		44.8	4.77	10.0	1.95		
0*		38.7	5.18	11.2	2.2		Dog 5: 7.2 grams citrate
0		32.0	5.25	10.7	2.4		[dog survived]
48		32.0	5.10	10.8	2.7		
106		39.3	5.23	10.8	2.1		
155		38.4	4.46	7.5	3.1		

* Before atropine.

from the length of the electrical ventricular systole, as expressed by "K" values is only slightly changed.

Equal elevation of serum phosphate cannot be attained by phosphate infusion in the unanesthetized dog for reasons already given. With nembutal narcosis, on the other hand, serum phosphate piles up on infusion and is accompanied by a corresponding fall in serum calcium with manifest tetanic muscular activity

and prolongation of the relative electrical ventricular systole. When citrate is infused the serum calcium behaves quite differently. Its total quantity does not fall, indeed it may be increased to 12 or even 14 mgm. per cent. Even so, the same tetanic muscular contractions occur and the prolongation of the relative electrical ventricular systole may be greater. These muscular and cardiac dysfunctions can be corrected promptly by the injection of small quantities of ionized calcium.

Differences in the loss of fluid and of protein through the capillary wall and the changes in the fractions of serum calcium included in this series of experiments are of interest in association with mechanisms concerned in capillary permeability. Phosphate infusion results in considerable loss of fluid from the plasma; when citrate is used the loss in fluid is small, in protein great. This provides further evidence that particular conditions determine the permeability of the capillary wall for different constituents of the plasma.

The decrease in total serum calcium and the concomitant hemoconcentration with phosphate infusion together with the increase in total calcium without loss of fluid from the plasma with citrate, support the theory that calcium is the determining factor for the passage of fluid through the intercellular "cement" substance of the capillary wall (9). The theory is weakened through the fact that the total calcium is not changed materially when shock is produced by the tourniquet method and the loss of fluid nevertheless is considerable.

Even if the interaction of calcium and cement substance was determining for the passage of fluid, this would not hold for protein. Its loss is maximal with citrate infusion when the total calcium is not reduced and indeed augmented. That calcium made dialysable by citrate (6) may be washed away from the cement substance during the citrate infusion to allow the passage of protein is incompatible with the fact that there is no associated hemoconcentration.

Available knowledge does not exclude passage of fluid through the cement substance; it does support the assumption that this is not likely for protein and that the capillary lining cells are involved. The hypothesis is strengthened by the fact that the loss of protein through the capillary wall with citrate is materially reduced by atropinization before infusion. Under these circumstances it would be more probable that neural control involves the lining cells of the capillary rather than the cement substance.

It can only be queried whether atropinization that allows survival after what would otherwise be more than twice the fatal quantity of citrate is dependent upon the known action of the drug as a definite coronary vasodilator (10).

SUMMARY

1. A rise in serum phosphate accompanies shock produced by tourniquets on the hind legs of dogs. No large changes occur in the total serum calcium or in the "K" values of the electrocardiogram.

2. Infusion of phosphate in the anesthetized animal is followed by increase in serum phosphate, loss of total serum calcium, fall in blood pressure, hemo-

concentration, tetanic muscular contractions, prolongation of the relative electrical ventricular systole (K value) and death.

3. Infusion of citrate in the anesthetized animal does not influence blood pressure until the terminal moments of life; only moderate hemoconcentration occurs but there is marked loss of plasma protein. The total serum calcium tends to rise even though tetanic muscular contractions and alteration of the electrical ventricular systole are outspoken.

4. The atropinized animal survives the infusion of more than twice the amount of citrate which is fatal without the use of this drug. The only other difference manifested is a decrease in the loss of plasma protein.

5. These findings provide a basis for consideration of the mechanisms involved in permeability of the capillary wall.

REFERENCES

(1) MYLON, E., M. C. WINTERNITZ, R. KATZENSTEIN AND G. J. DE SÜTÖ-NAGY. This Journal 137: 280, 1942.
(2) GOVIER, W. M. AND C. M. GREEN. J. Pharmacol. and Exper. Therap. 72: 318, 321, 1941.
(3) CARTER, E. P. AND E. C. ANDRUS. J. A. M. A. 78: 1922.
(4) KELLOG, F. AND W. J. KERR. Am. Heart J. 12: 346, 1936.
(5) BINGER, C. J. Pharmacol. and Exper. Therap. 10: 105, 1917.
(6) SHELLING, D. H. AND H. L. MARLOW. J. Biol. Chem. 78: 661, 1928.
(7) SCUDDER, J. Shock. Philadelphia, J. B. Lippincott & Co., 1940.
(8) KATZENSTEIN, R., E. MYLON AND M. C. WINTERNITZ. This Journal 139: 307, 1943.
(9) CHAMBERS, R. AND B. W. ZWEIFACH. J. Cell. and Comp. Physiol. 15: 255, 1940.
(10) LEROY, G. V., G. K. FENN AND N. C. GILBERT. Am. Heart J. 23: 637, 1941.

THE TOXICITY OF THORACIC DUCT FLUID AFTER RELEASE OF TOURNIQUETS APPLIED TO THE HIND LEGS OF DOGS FOR THE PRODUCTION OF SHOCK[1]

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This study was designed to explore the effects of injection of thoracic duct fluid, collected promptly after the release of tourniquets applied to the hind legs of dogs. Some of the premises for the undertaking include the marked rise in serum phosphate (1) and potassium (2) found in the same circumstances. On the basis that these electrolytes are derived from the cellular elements of the legs distal to the tourniquets and that they are evidence of disintegration, it was thought likely that other materials, including protein derivatives or degradation products, also might be released and be absorbed from the tissues through the lymph.

Study of the blood from such extremities has not been rewarding (3) and results concerning the effects of extracts of the tissues of the leg are conflicting (4). On the other hand, it has been shown that taping of the legs before or immediately after release of the tourniquets reduces the mortality (5). Such taping may prevent loss of fluid into the damaged tissues at the site of application and distal to the tourniquets and also absorption of toxic substances through the lymphatics. Freezing of the legs during the period of ligation has similar effects (6).

The first results of this study were not convincing. They became more satisfactory when it was realized that blood pressure changes and other major evidences of shock, occasionally leading to death, might follow nothing more than nembutal anesthesia.

Following the description of methods several series of experiments will be reported. These indicate differences in the reactions to nembutal as compared to thoracic duct fluid and also differences in the reactions to such fluids from dogs with and without preliminary use of tourniquets.

PROCEDURES. Shock was produced in dogs, after the administration of nembutal, by the application of tourniquets as high as possible on both hind legs. The tourniquets were applied not only to occlude the circulation of the extremities but tightly enough to bruise the underlying soft tissues. After five hours the thoracic duct was exposed as follows:

The median border of the left jugular vein was brought into view by an incision extending upward from the sternal notch along the edge of the left sternocleidomastoid muscle. The vein was followed to its juncture with the left subclavian. When the latter was elevated the thoracic duct became visible

as did the larger tributaries, usually two, that join to form a sac emptying posteriorly into the vein. Constriction of this vein on the cardiac side of the entrance of the lymph sac caused the ducts to become engorged so that they could be differentiated readily by their color from veins. After ligation of the ducts from the left front leg and from the head, the thoracic duct was incised a centimeter below the lymph sac to avoid valves that might complicate canalization.

The duct collapses as soon as it is cut and the opening, made preferably with small sharp scissors, should be kept in sight constantly until the cannula (a glass tube with a bore of 1.5 mm.) is fixed in place. It is important not to use force for insertion of the cannula. Lymph usually begins to well out of the free end of the cannula promptly. When this does not occur suction should be avoided as it will collapse the thin-walled duct. Introduction of 0.5 cc. of air through the mouth of the cannula is helpful in causing a free flow. The lymph from a normal dog, not fed for 12 hrs., usually is opalescent, rarely milky; it becomes pink and at times frankly blood-stained within a few minutes after release of tourniquets applied to the extremities.

The lymph was collected from the cannula in a heparin-containing tube immersed in a salt-ice mixture. Its rate of flow varied and was more rapid in young animals. Usually up to 20 cc. were collected in from $\frac{1}{2}$ to 2 hrs. The experiments will be presented in two major groups differing from each other only in the amount of nembutal used for narcosis. Each of these major groups, to be designated as I and II, includes two series, one involving the use of thoracic duct fluid from normal dogs (A), the other thoracic duct fluid collected after release of tourniquets applied to the hind legs (B).

Group I. The animals included in this group received an initial intravenous injection of 30 mgm. of nembutal per kgm. body weight; when necessary 10 mgm. per kgm. body weight were given later in the experiment.

(A) *Normal thoracic duct fluid.* This series includes 28 dogs. Fourteen of these supplied the thoracic duct fluid for injection into the other fourteen. In each instance the fluid for injection into an animal was derived from a single donor.

The recipients were small in contrast to the donors, which were selected for their large size. Like the donors they were anesthetized with 30 mgm. of nembutal per kgm. body weight. After the femoral blood pressure record was started, from 7 to 20 cc. of thoracic duct fluid were injected into a vein of the foreleg. The observations were discontinued if no change in the animal's blood pressure or general condition occurred within two hours. If a change occurred the records were continued until the blood pressure reached the pre-injection level.

All of the animals survived the procedure and subsequently developed no untoward signs. Half of them showed no change in blood pressure. The other half had a fall in pressure, which was gradual, varying in onset from 10 to 70 min. after injection of lymph; lasting from 40 to 300 min. and representing a drop from 30 to 70 mm. Hg. The time lapse between injection and beginning

of the fall in pressure, its duration and extent do not bear any relation to each other or to the amount of lymph injected. These facts are included in table 1.

(B) *Thoracic duct fluid from animals in shock.* This series includes 36 animals divided equally into donors and normal recipients. Twelve of the latter, 66 per cent, showed a fall in blood pressure as compared to 50 per cent in series "A". The fall was gradual, varying in onset from 10 to 60 min. after the injection of lymph, lasting from 45 to 240 min. and representing a drop of from 45 to 100 mm. Hg. Three of the animals died within a few hours. The fall in blood pressure showed only a slight rise before death. The other nine dogs recovered. The amount of lymph varied from 7 cc. to 51 cc., and it is interesting that the animal receiving the smaller dose was one of the three which died. The results are summarized in table 2.

Group II differs from the one just presented in: 1. The amount of nembutal used for narcosis. 2. The manner of its administration. 3. Preliminary ad-

TABLE 1

Effect of lymph from normal dogs (30 mgm. of nembutal per kgm. body weight)

DOG	BLOOD PRES- SURE BEFORE INJECTION	ONSET OF FALL (TIME AFTER INJECTION)	DURATION	EXTENT	AMOUNT OF LYMPH INJECTED
	mm. Hg	*min.*	*min.*	*mm. Hg*	*cc.*
1-7		none	0	0	10, 16, 12, 11, 16, 14, 20 respectively
8	150	10	120	50	12
9	105	15	240	60	17
10	70	20	120	30	11
11	110	40	300	60	18
12	100	45	40	45	10
13	120	65	210	70	9
14	130	70	120	50	8

ministration of the anesthetic several days prior to the experiment with recording of blood pressure.

These precautions were deemed essential as it was found that a fall in blood pressure may occur with the larger dose of nembutal even though no other procedure is involved. Occasionally an animal may even die.

The initial quantity of nembutal given to all of the animals included in group II, both donors and recipients, was 25 mgm. as distinct from 30 mgm. per kgm. body weight for group I. When subsequent injection of the drug was necessary on account of the length of an experiment 2 mgm. were used as distinct from 10 mgm. per kgm. body weight for group I. Care was exercised to inject the nembutal saline solution (10 mgm. per cc.) slowly and uniformly over a period of 2 min.

The reactions of many of the animals in both series "A" and "B" to this amount of nembutal were determined days before the final experiment. In no instance was there any fall in blood pressure, with the lighter anesthesia. There

is, in fact, a tendency to a slight rise in pressure. This control reaction is recorded for the animals in which it was used.

TABLE 2

Effect of lymph of dogs in shock (30 mgm. of nembutal per kgm. body weight)

DOG NO.	BLOOD PRESSURE BEFORE INJECTION	ONSET OF FALL	DURATION	EXTENT	AMOUNT OF LYMPH INJECTED
	mm. Hg	min.	min.	mm. grad.	cc.
1-6		none	0	0	9, 17, 7, 10, 9, 18 respectively
7	115	10	45	40	15
8	90	10	120	50	13
9	90	10	40	50	12
10	110	13	45	40	13
11	120	15	120	55	9
12	130	20	120	90*	12
13	130	30	75	85	7
14	130	30	160	90	11
15	95	30	120	50	10
16	140	40	240	100	9
17	110	55	120	55	7
18	115	60	120	35	15

* Died.
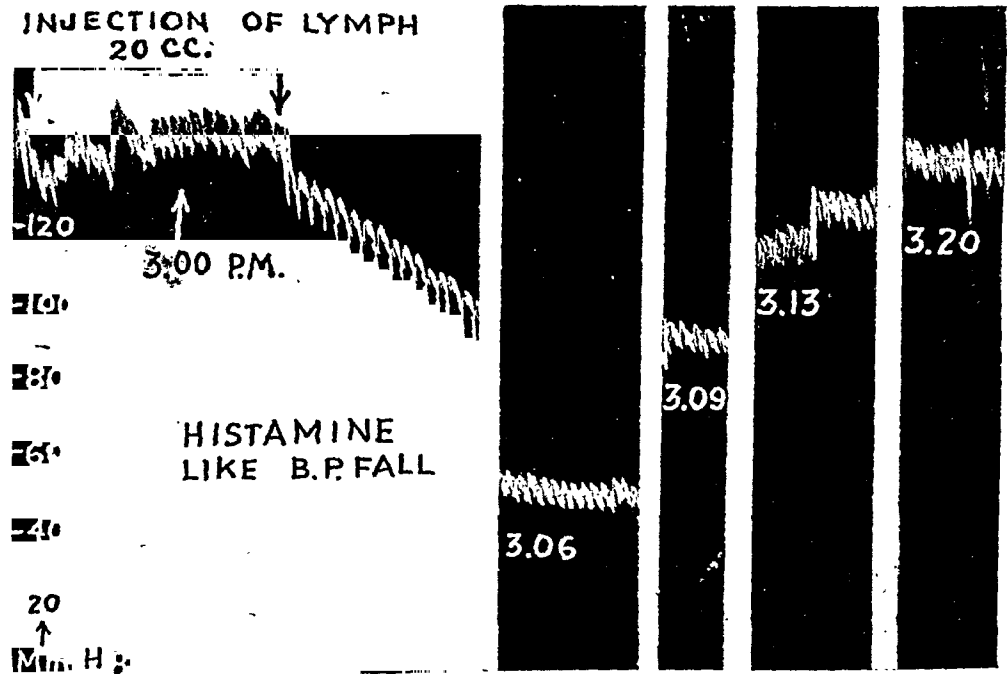
Fig. 1. Fall of blood pressure following the injection of lymph from a dog in shock. The curve resembles that after the injection of small doses of histamine.

A. *Normal thoracic duct fluid.* The series includes 20 animals divided equally into donors and recipients.

The experiment involved the intravenous injection of thoracic duct fluid from a separate donor for each recipient, as described for the previous series. Only
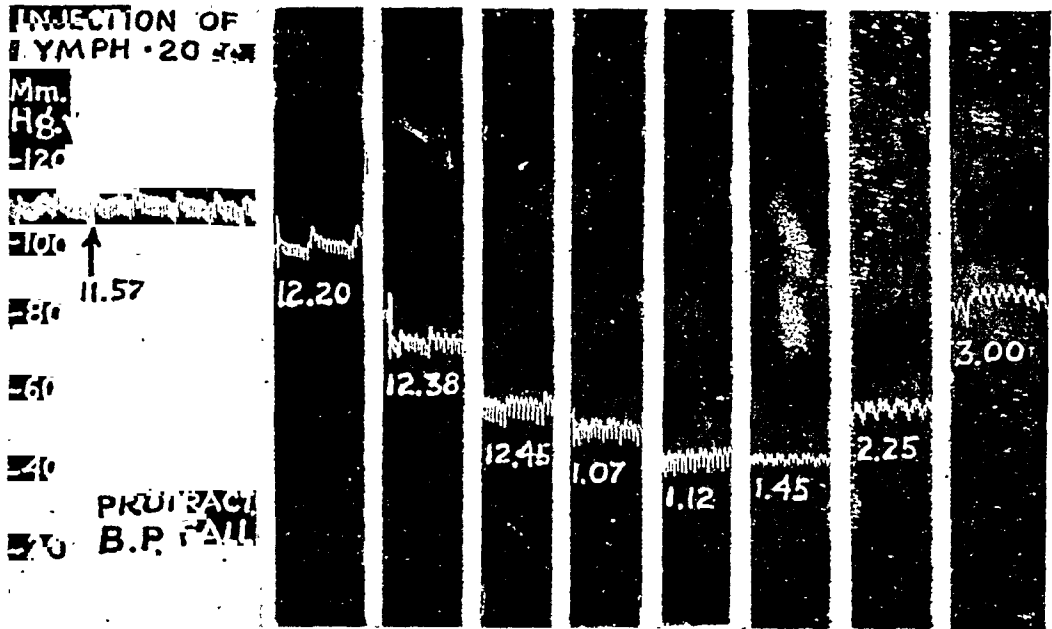
Fig. 2. Fall of blood pressure following the injection of lymph from a dog in shock. In contrast to figure 1 the onset is delayed, the depression is gradual and the blood pressure has not quite returned to its original level after 155 min.

TABLE 3

Effect of lymph of dogs in shock (25 mgm. of nembutal)

DOG NO.	BLOOD PRESSURE BEFORE INJECTION	ONSET OF FALL	DURATION	EXTENT	AMOUNT OF LYMPH INJECTED
	mm. Hg	minutes	minutes	mm. Hg	cc.
1–8*		0	0	0	25, 19, 13, 20, 12, 16, 20, 7 respectively
9*	145	Immediate	6†	90	7
10*	145	Immediate	8†	80	28
11	130	Immediate	8†	80	20
12*	140	1	36‡	30	15
13	140	2	13†	90	20
14	120	3	180‡	120	13
15*	155	5	120‡	80	15
16*	140	6	40‡	90	14
17	105	16	90‡	26	16
18	105	25	155‡	58	20

* Preliminary determination of the effect of nembutal.
† Sharp drop.
‡ Gradual drop.

one of the recipients showed any change in blood pressure. A fall began 1 min. after the injection of 20 cc. of lymph, gradually reached its maximum of 35

mm. Hg, and returned to the pre-injection level all within the next 3 min.   Three of the other nine animals that showed no reaction also received 20 cc. of lymph.

B. *Thoracic duct fluid from animals in shock.*   This series is made up of 36 dogs divided equally between donors and recipients.   Of the latter, ten were tested some days previously for their reaction to the smaller dose of nembutal. They showed no change in blood pressure and no demonstrable general effect either during the narcosis or in the following days prior to the experimental procedure.

Nine of the 18 recipients showed a fall in blood pressure.   In four this resembled the response to the injection of small amounts of histamine.

Figure 1 represents the record of one of these dogs.   The fall was fairly sharp and promptly followed the injection of the thoracic duct fluid; its duration varied from 6 to 13 min. and its extent from 86 to 96 mm. Hg.

The response of the other 5 animals was represented by a smooth curve.   The fall of pressure began less promptly, 1 to 25 min. after the injection of thoracic duct fluid and continued for a considerably longer period, from 36 to 120 min., in the animals that recovered (fig. 2).

This sequence of events occurred in all 5 animals; in one, however, the pressure failed to rise again and the animal died.   The amount of lymph used for the individual dog varied from 7 to 25 cc.   As can be seen from the table that follows, the dose of lymph is not related to the type of curve or the extent of the fall in pressure (table 3).

SUMMARY

The thoracic duct fluid becomes blood-stained after the release of tourniquets applied to the hind legs of dogs.   When this fluid is injected intravenously its effect upon blood pressure is variable.   Injection of as little as 7 cc. may be associated with a severe and protracted fall in blood pressure which may terminate in the death of the animal.

The vasodepressor effect is more frequent when large doses of nembutal have been used for narcosis.

Injection of such amounts of nembutal may be associated with a fall in blood pressure even when no other procedure is involved.

When narcosis is controlled to avoid vasodepression injection of thoracic duct fluid from normal dogs has no significant effect.

In contrast, a fall in blood pressure follows in 50 per cent of the animals injected with the thoracic duct fluid of animals shocked by the tourniquet method.[2]

REFERENCES

(1) MYLON, E., M. C. WINTERNITZ AND G. J. DE SÜTÖ-NAGY.   This Journal **139**: 299, 1943.
(2) SCUDDER, J.   Shock.   J. B. Lippincott, Philadelphia, 1940.
(3) HOLT, R. L. AND A. D. MACDONALD.   Brit. Med. J. **1**: 1070, 1934.
(4) WILSON, H. AND N. W. ROOME.   Arch. Surg. **32**: 334, 1936.
(5) MYLON, E., M. C. WINTERNITZ AND G. J. DE SÜTÖ-NAGY.   This Journal **139**: 313, 1943.
(6) DUNCAN, G. W. AND A. BLALOCK.   Arch. Surg. **45**: 183, 1942.

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² These findings are in accord with those of Doctor A. Blalock (personal communication).

STUDIES ON THERAPY IN TRAUMATIC SHOCK[1]

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The approach to the study of therapy in shock selected for this investigation was the well known application of tourniquets to the hind legs of dogs (1, 2, 3). This method of inducing characteristic manifestations of the condition was chosen on the basis of experience with the procedure modified, as will be seen, to provide a standard that yielded a satisfactorily uniform clinical picture as a measure of therapy.

The therapy was devised to determine the relative importance of well recognized factors like the circulating blood volume (4) as this is affected by the content of the capillary bed, capillary permeability, etc. Particular attention was directed toward the effect of plasma infusion as compared to plasma-albumin solutions with similar colloidal osmotic pressure. In addition, methylene blue and succinate were used in order to compare their known action on tissue metabolism with the effect of solutions exerting a high colloidal osmotic pressure.

Without doubt there are many stages in the development of shock that may rectify themselves or be corrected by agents designed to compensate for manifestations of inadequate circulation. Long experience with various substances—culminating with serum albumin—allows no other conclusion.

These positive results, however, leave the question open concerning the stages of shock in which such therapy is not adequate. Then queries arise concerning the extent to which impaired circulation may be responsible for metabolic changes like inhibition of cell respiration, alteration of glycolysis or glyconeogenesis. Such deficiency may follow other and more direct cause than impaired circulation and be the basis for the theory of the toxic genesis of traumatic shock.

The presentation that follows deals first with the problems underlying the adoption of the particular procedure utilized for the production of shock and then with a series of therapies designed to ascertain the relative importance of the mechanical circulatory disturbances as distinct from those associated with changed metabolism.

PROCEDURE. The method used in this study for the production of shock and its therapy was developed with particular consideration of the influence of various factors known to affect the responses of animals. They include nembutal anesthesia, repeated withdrawal of blood for analyses during the course of the individual experiment, the application of the tourniquets to the legs with particular reference to the amount of underlying muscle and the traumatization effected through the pressure exerted by the tourniquet and finally the local

swelling in the traumatized area associated with local edema. Each of these problems will be presented now.

It is well known that the anesthetic nembutal also exerts profound side effects,—the respiratory rate is markedly reduced, the blood pressure falls, there is hemoconcentration with loss of protein as well as fluid from the blood, the renal function is suppressed and the body temperature falls. The picture in its totality is difficult to differentiate from shock produced in other ways; it may actually terminate with the death of the animal. While there are undoubtedly individual variations in the response to nembutal on the part of animals, the major factors are the size of the dose and the method of its administration.

A dose of 30 mgm. per kgm. introduced intravenously and slowly is tolerated without serious manifestation by the majority, but one out of four animals will develop more or less serious signs of the variety enumerated above that would confuse the interpretation of further procedure in the production of shock. This is avoided when 25 mgm. is the dose.

A complicating factor is the long period of anesthesia required by the tourniquet method of producing shock. Even 30 mgm. per kgm. usually requires reinjection of smaller doses after several hours. The amount of the drug used for reinjection must be small to avoid untoward signs: 10 mgm. per kgm. is an excess, 5 is adequate and this often may be reduced to 2 mgm. per kgm. The procedure adopted therefore consists of an initial dose of 25 mgm. per kgm. followed as required after several hours by reinjection usually of 2 mgm. per kgm. even though at times this has to be repeated.

The smaller initial and repeat doses of nembutal were selected because the blood pressure tended to rise in contrast to the tendency to fall with the larger doses. The blood pressure proved to be the most satisfactory indicator of the general condition of the animal as influenced by nembutal.

Another reason for the selection of the quantity of nembutal is the fact that the smaller doses exert less influence than the larger amount on the circulating blood volume and any tendency to a reduction in this volume must be avoided. This is evidenced since even the withdrawal of blood for essential analyses in control experiments facilitated the development of shock. As would be expected, the more blood withdrawn from the circulation, the greater the effect. With this in mind, no blood was withdrawn in this study as the objective was the development of shock by the use of the tourniquet method, and its therapy, with elimination, so far as possible, of contributory influences.

As soon as anesthesia was evident the tourniquets were applied to the back legs. Care was taken in the selection of the rubber tubing, the place of application and the tension exerted. Pure gum hand made tubing was used with a bore of $\frac{5}{16}$ inch and a wall of $\frac{1}{16}$ inch with maximal elasticity and tensile strength indicated by the fact that it was able to suspend a 75-pound weight. It was applied as high as possible on the thigh and fully extended so that it would exert a maximal pressure on the underlying musculature. This was found to be necessary as mere occlusion of the circulation is not followed by shock in a sufficiently high and constant percentage of animals.

When the tourniquet is applied not only to occlude the circulation but to exert a maximal pressure on the soft tissues of the thigh as manifested by hemorrhage in the muscle and even in the skin adjoining the groove made by the rubber tube, the release of the tourniquet is followed promptly and constantly by brawny induration of the leg. This begins as a rule in the inner thigh, extends circularly and distally and attains its height in one hour. By this time the blood pressure has dropped markedly and is approaching the shock level, providing, with sufficient speed and constancy, opportunity for the study of therapy.

As has been pointed out, the method of applying the tourniquet is a factor in the swelling of the extremity after the pressure is released. With only vascular occlusion there is little edema of the leg; with traumatization of the soft tissues the edema is marked. As will be seen even the more extensive swelling of the leg that occurs when the tourniquet has been applied as tightly as possible is greatly increased if the animal lives for a few hours and has had the circulating fluid of its blood replenished with saline or glucose. To avoid this complication as a factor in the outcome, the legs were firmly taped as high as the ilio-femoral joint and including the buttocks, thus limiting further local loss of fluid. The right leg was taped one hour after the release of the tourniquet at a time when, as has been said, the pressure had approached the shock level; the left was taped one hour later. This delay was necessitated by the fact that the left femoral artery was used to make the kymograph records as it was deemed desirable to avoid structures in the neck (carotid sinus) that might influence the reactivity of the animal.

Therapy was instituted promptly after taping of the right leg—one hour after release of the tourniquet. It consisted of the intravenous administration of from 325 to 450 cc. of different fluids introduced into a vein of the foreleg for one hour by the drip method. This was discontinued with the removal of the femoral cannula and the taping of the left leg.

SUMMARY OF PROCEDURE. 1. Intravenous administration of nembutal 25 mgm./kgm. followed if necessary after several hours with 2–4 mgm./kgm. 2. Application of tourniquets to both hind legs. 3. Release of tourniquet from left leg after five hours followed by passive motion of the leg for a few seconds. 4. Ten minutes later cannulation of left femoral artery for blood pressure record. 5. Release of the tourniquet from the right leg followed by passive motion. 6. One hour later: taping of right leg and institution of intravenous therapy. 7. One hour later: discontinuance of intravenous therapy. 8. Removal of cannula from left femoral artery and closure of wound with sulfathiazole powder dressing. 9. Taping of left leg. 10. Removal of tapes from both legs after 24 hrs.

The animals were protected from cold and kept at about 21°C. Survival beyond 48 hrs. was chosen arbitrarily as the end point but it should be recorded that animals that lived for this period very rarely died without cause quite independent from shock.

EXPERIMENTS. The experiments may be grouped into the following categories and will be presented in this order. The fundamental procedures are the

same for all groups. Two primary variants only occur. They involve taping of the legs and intravenous therapy. Group I is a control series without taping of legs or therapy of any variety. The legs were taped immediately after release of the tourniquets in group II. In all the others the order of procedure indicated above was followed in detail.

Group I. Control—legs were not taped and no therapy was used. Group II. Legs were taped immediately after release of the tourniquets; on some animals the blood pressure was not recorded. No therapy was used. Group III. Taping alone as indicated in procedure, without supporting therapy. Group IV. Taping and intravenous saline. Group V. Taping and intravenous dog-plasma. Group VI. Taping and intravenous dog-plasma-albumin. Group VII. Taping and intravenous dog-plasma-globulin. Group VIII. Taping and intravenous dialyzed dog-plasma. Group IX. Taping and intravenous pre-

TABLE 1

Shows the survival time of 48 dogs after release of tourniquets applied to both hind legs for 5 hours

SURVIVAL TIME	NUMBER OF DOGS	PERCENTAGE
hours		
1– 1¾	6	12.5
2– 2¾	12	25.0
3– 3¾	8	16.7
4– 4¾	6	12.5
5– 5¾	3	6.3
7– 7¾	3	6.3
11–11¾	3	6.3
13–13¾	2	4.2
15	2	4.2
19	1	2.0
35	1	2.0
48+	1	2.0

Average survival time of 47 animals 5.7 hours.

viously alkalinized dog-plasma. Group X. Taping and intravenous saline plus methylene blue. Group XI. Taping and intravenous dog-plasma-albumin plus methylene blue. Group XII. Taping and intravenous saline plus succinate.

Group I. It should be emphasized that long experience was essential for the development of the procedure outlined above and necessary to secure just those details required for a rapid development of signs of shock that would have a fatal outcome within a few hours without effective therapy. These preliminaries are not included in the tabulated results that follow. Further, the control group was not completed as a unit, but accumulated gradually with small numbers of animals sandwiched in as the therapeutic variants were studied.

This group now comprises 48 dogs included in table 1.

Forty-seven of the 48 dogs died within the arbitrarily chosen limiting time of 48 hrs. Although the survival periods are spread over a larger time interval,

35 or 73 per cent of all animals died during the first $5\frac{3}{4}$ hrs., an average survival time of 3 hrs. Six of these 35 dogs or $12\frac{1}{2}$ per cent died within $1\frac{3}{4}$ hrs. after release of the tourniquet, too rapidly for the institution of therapy. The impression grew that this group with rapid fall of blood pressure to shock level within an hour more or less also developed early bradycardia and complete irregularity far more frequently than those that survived for a longer time.

When these 6 dogs or $12\frac{1}{2}$ per cent are discarded, there remain 29 animals, or 60.5 per cent, that survived 2 to $5\frac{3}{4}$ hrs. after the release of the tourniquets. The blood pressure in these began to fall promptly but did not reach the low level so quickly. It averaged 70–85 mm. Hg after 1 or $1\frac{1}{4}$ hrs. and without therapy the pressure continued to fall.

The remaining 12 animals or 25 per cent had a longer survival period. The large majority of these showed a more gradual drop in blood pressure.

Group II includes 9 animals whose legs were taped immediately on release of the tourniquets from the hind legs. Three animals survived indefinitely. The average survival time for the remaining six was 32+ hrs., the shortest 16 hrs.

There can be little question that immediate taping of the legs after release of the tourniquets plays an important part in the length of survival of the animal. This is in accord with findings of Duncan and Blalock (5).

Group III comprises 9 animals whose hind legs were taped as indicated under procedure. The survival time as compared with that of group I where the legs were not taped is not significantly changed. It averaged $5\frac{2}{3}$ hrs., the spread for individual dogs approximated that of the control group closely and no beneficial effects can be attributed to this procedure alone.

Group IV includes ten animals whose legs were taped after the release of the tourniquets in accordance with the adopted procedure and who received saline intravenously in amounts equal to about 4 per cent of the body weight.

Only one of these ten animals survived indefinitely. It is of interest to note that the shortest survival time was 5 hrs., and the average 12+ hrs. as compared to the control group in which the shortest time was less than 1 hr. and the average 5.7 hrs.

The fall in blood pressure often was marked within the first hour after the release of the tourniquets. In some instances it continued to fall, in others it maintained the same level, while in still others it rose slightly during the infusion. Moreover, these variations in the blood pressure were not related to the survival time.

Group V includes 18 animals that received freshly prepared, pooled, citrated, dog plasma one hour after release of the tourniquets on the hind legs. Table 2 indicates the amount of plasma infusion—in general accord with the body weight—and the fate of the dog.

Only four of the 18 dogs failed to survive the arbitrary figure of 48 hrs. Three of these manifested changes at autopsy that might well have been expressions of other important causes than shock as contributing to their death. The other 14 animals survived the 48 hr. limiting period and may well have lived on indefinitely. This is the more remarkable since the cannulated and tourniquetted

left leg in contrast to the right developed dry gangrene with time in a number of the dogs and provided an excellent nidus for infection.

The value of the plasma began to be manifested after 100± cc. had been infused. Although emphasis can not be laid on this observation the blood pressure began to rise; the cardiac action, often slightly irregular preceding infusion, became stronger and regular; the anesthesia became increasingly less and usually no longer evident by the time the infusion was discontinued. In the next four hours the dogs sat up and took water when this was offered. The record is typical.

TABLE 2

The influence of dog-plasma infusion on the survival time

DOG NO.	WEIGHT	TOTAL AMOUNT OF DOG PLASMA USED	SURVIVAL TIME	REMARKS
	kgm.	*cc.*	*hours*	
1	12	350	∞	
2	10.9	360	∞	
3	12.3	360	∞	
4	9.1	300	∞	
5	9.5	340	∞	
6	12	300	∞	
7	11.4	390	25	Fulminating infection of leg with cl. Welchii
8	13.5	430	∞	
9	9.1	400	38	Pneumonia
10	9.1	400	∞	
11	10.9	390	∞	
12	9.1	350	15	Hemorrhages of the myocardium involving His bundle
13	6.1	350	∞	
14	7.5	490	∞	
15	11.4	400	17	No lesions found at autopsy
16	6.8	350	∞	
17	7.7	400	∞	
18	8.4	350	∞	

Group VI includes 12 dogs that received dog-plasma-albumin one hour after the release of the tourniquets. The plasma-albumin was prepared by saturation of the plasma with ammonium sulfate after the globulins had been removed by half saturation with the same agent. The ammonium sulfate albumin-precipitate was dialyzed first against tap water for one day at room temperature, then against distilled water for two days and finally against saline for two days, both at +4°C. Only traces of ammonium sulfate were detectable after final dialysis. The protein of the albumin solution was determined by the Kjeldahl method and brought to a content of 3.9 to 4.7 per cent to compare with the

colloid osmotic pressure of the plasma.[2] Table 3 shows the weight of the animals, the per cent of albumin and the amount of the infusion fluid as well as the result of the experiment. Only one of these 12 dogs survived for 46 hrs.— one for 24 hrs. and all the rest less than 17 hrs. The average was 16 hrs., 4 hrs. longer than those that were infused with saline. The group includes several animals in which the blood pressure had only fallen slightly, to 85 or 90 mm. Hg when the infusion was started, and had been maintained at this level during
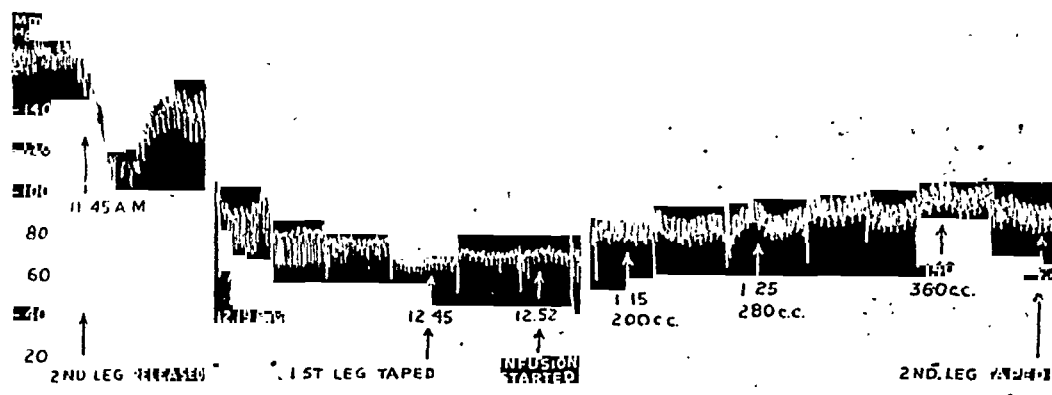
Fig. 1. Blood pressure changes following the release of tourniquets occluding the hind legs of dog 2 of group 5, table 2. Therapy: Taping of legs plus dog plasma infusions.

TABLE 3

The influence of dog-plasma-albumin infusion on the survival time

DOG NO.	WEIGHT	PERCENTAGE OF ALBUMIN SOL. USED	TOTAL AMOUNT OF ALBUMIN SOL. USED	SURVIVAL TIME
	kgm.		*cc.*	*hours*
1	9.5	4.45	340	15
2	14.5	4.45	350	24
3	11.4	4.45	540	14
4	16.4	4.75	400	8
5	13.6	4.75	460	14
6	8.2	4.75	320	14
7	13.6	3.9	480	14
8	13.6	3.9	470	12
9	14.1	3.9	470	46
10	14.1	4.0	400	4
11	10.0	4.0	350	17
12	6.8	4.0	275	13

the following hour while the record was in progress. This is illustrated in the curve of dog 6, table 3.

Group VII. The nine animals in this group received infusions of dog-plasma-globulin after the release of the tourniquets. The globulin was prepared by half saturation with ammonium sulfate; the resulting precipitate was dialyzed

[2] It is assumed that at equal molar concentrations the colloid-osmotic pressure (6) of albumin is at least 2.4 times larger than of globulin.

against tap water for a day at room temperature and then against 2 per cent saline for three days at 4°C. The protein content of the solution was ascertained by aid of the Kjeldahl method and brought to 4.75–5 per cent concentration with 1 per cent saline. One of these nine animals survived indefinitely, the remaining eight died in from 4–22 hrs.—an average of 8.7 hrs. This compares unfavorably with albumin with a survival period of 16+ hrs. and even with saline with 12+ hrs.

Group VIII includes eight animals infused with dialyzed dog-plasma 1 hr. after the release of the tourniquets. The dialysis of pooled dog-plasma was effected in closed cellophane sacs against saline at 4°C. for 8–10 days.

In contrast to undialyzed plasma only one of these eight animals survived indefinitely. The others had an average survival period of 9½ hrs. spread between 5 and 17 hrs. This does not compare favorably even with the results after saline infusion. It indicates clearly that plasma loses some important factor through dialysis as this was carried out.
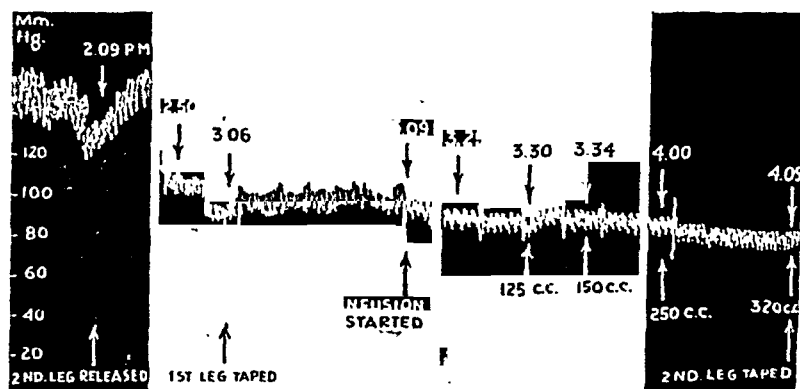
Fig. 2. Blood pressure changes following the release of tourniquets occluding the hind legs of dog 6 of group 6, table 3. Therapy: Taping of legs plus infusion with dog plasma albumin.

Group IX includes 8 dogs infused with dog plasma. This was alkalinized with sodium hydroxide to pH 9.9–10.9, allowed to remain so at +4°C. for 3 days and then return to pH 7.3 with hydrochloric acid. This plasma was not comparably efficacious in saving the lives of the animals. Two dogs did survive indefinitely but the other 6 all died within 19 hrs., an average of 11⅓ hrs. One of the survivors received plasma that had been alkalinized to pH 9.9 and one to pH 10.9. This indicated that the unfavorable influences exerted by the alkali on the plasma are manifest at the lower pH level.

Group X consists of 9 dogs infused with saline supplemented with from two to three intravenous injections of 5 cc. of 0.5 per cent methylene blue in saline during the course of the 1 hr. of therapy.

The use of methylene blue was based upon the hypothesis that interference with tissue respiration may result in changes against which the hydrogen receptor properties this dye offered might have advantages. The effect it exerts on blood pressure also was deemed desirable even if the manner in which this is

brought about is not clear. In accord with previous experience it was found that methylene blue injection was followed invariably by a rise in blood pressure. If 50 mgm. of the dye are injected rapidly in 0.5 per cent concentration, the rise is acute and varies between 10 and 30 mm. Hg. When the injection is repeated
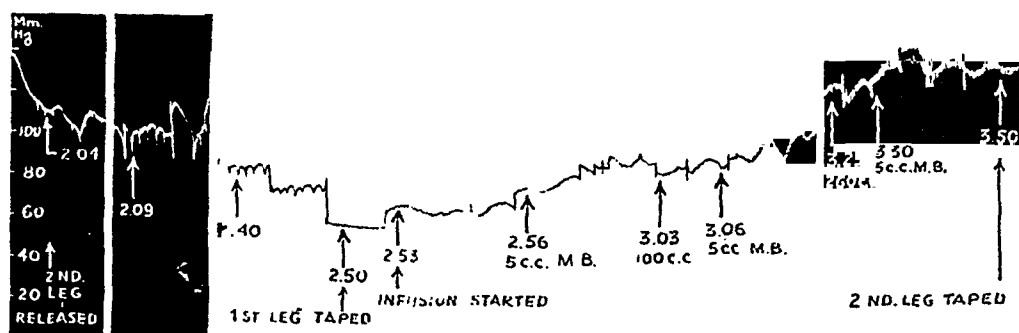
Fig. 3. Blood pressure changes following the release of tourniquets occluding the hind legs of dog 13 of group 11, table 4. Therapy: Taping of legs plus infusion of dog plasma albumin and injection of methylene blue.

TABLE 4

The influence of infusion with dog-plasma-albumin plus methylene blue on the survival time

DOG NO.	WEIGHT	TOTAL AMOUNT OF ALBUMIN SOL. USED	AMOUNT OF METHYLENE BLUE	SURVIVAL TIME
	kgm.	*cc.*	*mgm.*	*hours*
1	6.8	275	50	∞
2	7.7	290	70	∞
3	6.4	250	60	∞
4	6.6	270	55	5
5	15.0	410	50	∞
6	11.8	375	75	13
7	15.0	390	90	8
8	11.4	380	90	16
9	6.8	300	60	∞
10	8.0	350	75	40
11	7.3	350	65	∞
12	8.6	325	75	6
13	8.0	325	75	∞
14	6.8	450	75	12
15	7.7	400	75	38
16	10.0	440	80	7
17	7.7	400	70	22
18	8.6	400	75	19
19	7.7	300	40	∞

five or ten minutes later the response is of the same order of magnitude. Only one dog survived and this animal did not have a marked fall in pressure before therapy was started. This probably would have occurred had there been further delay in treatment. The other seven dogs had a spread of from 3 to 36 hrs. in survival time.

Group XI. These animals were subjected to the same procedures used in the preceding group with one exception. They were infused with dog-plasma-albumin instead of saline. The methylene blue injections were followed by the response already detailed. This is illustrated in figure 3. The blood pressure response following the injection of methylene blue though invariably
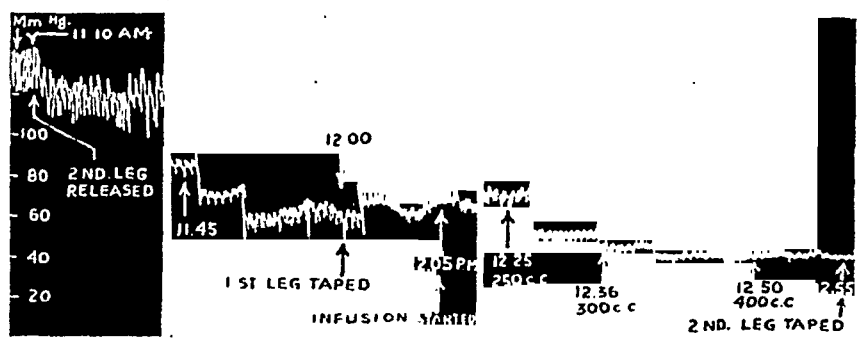
Fig. 4. Blood pressure changes gollowing the release of tourniquets occluding the hind legs of dog 11 of group 12, table 5. Therapy: Taping of legs plus infusion of saline containing succinate. Note the lack of immediate influence of the infusion on the blood pressure—and the survival of the animal.

TABLE 5

The influence of infusion with saline plus succinate on the survival time

DOG NO.	WEIGHT	TOTAL AMOUNT OF SALINE USED	SUCCINATE USED	SURVIVAL TIME
	kgm.	*cc.*	*mgm.*	*hours*
1	7.7	400	210	19
2	9.5	400	210	∞
3	8.6	400	150	∞
4	11.4	400	225	14
5	9.8	400	225	10
6	8.0	400	600	∞
7	8.2	400	600	∞
8	11.4	375	600	6
9	10.5	400	600	13
10	9.1	400	600	14
11	9.5	400	600	∞
12	8.2	400	600	16
13	8.4	400	1200	10
14	10.5	400	1200	12
15	10.9	500	1200	∞
16	8.2	500	1200	∞

present is not an absolute indication of the outcome of the experiment. Eight of the 19 dogs did survive, two of the remainder lived 38 and 40 hrs. respectively (table 4).

Group XII includes 16 dogs infused with saline supplemented with sodium succinate adjusted with hydrochloric acid to a pH of 7.3.

Succinate was used because of the recognized influence it exerts on the respira-

tion of surviving tissue. Whether it acts as a substrate bridging the interval when the tissue can not supply the necessary materials for oxidation or whether it functions as catalyst in the respiratory chain is still undecided.[3] Seven of these 16 animals survived.

The blood pressure records of dogs 11 and 14 contrast remarkably and this is particularly interesting as dog 11, whose blood pressure had fallen from 140 mm. Hg before the release of the tourniquets to less than 50 mm. Hg when therapy was discontinued, recovered completely and survived, while dog 14 whose pressure was 134 mm. Hg just before release of the tourniquets, fell to 64 mm. Hg within 50 min. and then again rose to the pre-release level before therapy was discontinued, died within 12 hrs.

The remarkable effect of the succinate-reinforced saline infusion on the heart should receive special mention. The cardiac contractions in most instances rapidly grew stronger and more regular as the infusion progressed and then a few hours after therapy was discontinued the behavior of the dogs in general was much more like those treated with plasma than after other therapies used, including albumin. Many sat up in their cages, accepted fluid and in general responded to the influences of their environment (table 5).

SUMMARY

1. A procedure is described that leads to "shock" with its characteristic manifestations of drop in blood pressure, hemoconcentration, loss of plasma protein and further in the death of 97 to 98 per cent of the dogs subjected to it. To attain this result the tourniquets must be applied not only to shut off the circulation of the hind legs but tightly enough actually to damage the underlying soft tissues.

2. When the legs are taped before or immediately after release of the tourniquets the incidence of shock is materially reduced and even when signs develop many animals ultimately survive.

3. The processes active in the damaged tissues during the first hour after the restoration of the circulation, including the local loss of fluid, are adequate to initiate fatal shock. Interference with these by taping the legs one hour after the release of the tourniquets is followed by a slightly longer survival period but not by increased recovery.

4. Therapy may be instituted at the end of one hour after the release of the tourniquets on the hind legs. At this time the animals are divisible into three groups on the basis of the severity of their symptoms. Twelve and a half per cent of the group are in an agonal state; 25 per cent do not yet show adequate signs of shock—the remaining 60.5 per cent have a marked fall in blood pressure and without treatment would die within a few hours. This latter group was chosen for therapy.

5. Restoration of the fluid content of the blood by intravenous infusion of

saline increases the survival period but leads to recovery only in one out of 10 animals.

6. Intravenous infusion of dog-plasma-albumin of a colloid-osmotic pressure equivalent at least to that of the total plasma further prolongs the survival period but does not influence recovery.

7. Globulin preparations of 5 per cent concentration from dog-plasma are not as effective as saline.

8. Citrated dog-plasma led to complete recovery in 14 out of 18 dogs. The four animals that died had a long survival period and 3 of them showed adequate other cause than shock for death.

9. Dog-plasma dialyzed as described is no longer effective as a therapeutic agent.

10. Dog-plasma alkalinized and readjusted to the original hydrogen concentration is no longer effective therapeutically.

11. Methylene blue administered intravenously promptly raises the blood pressure of an animal in shock. As a supplement to intravenous saline infusion it neither increases survival time nor percentage recovery.

12. Methylene blue as a supplement to dog-plasma albumin infusion prolongs the survival period and raises recovery rate to 42 per cent.

13. Sodium succinate, injected intravenously, is followed temporarily by more forcible and more regular cardiac action. As a supplement to saline infusion it results in a recovery of 43 per cent of the animals.

14. The beneficial influence of succinate contrasts to the known ineffectiveness of glucose. This may indicate a definite disturbance in the earlier stages of the carbohydrate breakdown. It is not known whether the phosphorus turnover is involved.

15. The therapeutic value of plasma is not fully explained by its colloidal osmotic pressure. The search for labile substances in plasma which restore metabolic processes is indicated.

REFERENCES

(1) WILSON, H. AND N. W. ROOME. Arch. Surg. 32: 334, 1936.
(2) ALLEN, F. M. Arch. Surg. 38: 155, 1939.
(3) MYLON, E., M. C. WINTERNITZ, R. KATZENSTEIN AND G. J. DE SÜTÖ-NAGY. This Journal 137: 280, 1942.
(4) JOHNSON, G. L. AND A. BLALOCK. Arch. Surg. 22: 626, 1931.
(5) DUNCAN, G. W. AND A. BLALOCK. Annals of Surg. 115: 684, 1942.
(6) KEYS, A. J. Physic. Chem. 42: 11, 1938.

POTENCY OF LIVER EXTRACT IN STIMULATING GASTRIC SECRETION BY INTRAVENOUS INJECTION AND BY DIRECT LAVAGE

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The possibility of a humoral or hormonal mechanism for the gastric secretory response to a meal was suggested by Edkins in 1906 (1). Subsequent work has furnished corroborative evidence for the existence of such a humoral mechanism (2, 3, 4, 5).

Kim and Ivy (6) obtained vaso-depressor free extracts of liver which contained potent secretagogues for gastric secretion. They also found that an isolated vagotomized pouch of the entire stomach could be made to secrete free acid by the slow intravenous injection of the extract, as well as by direct application to the gastric mucosa. According to their report, 400 mgm. of the extract was required to obtain a minimal response via the intravenous route while only 100 mgm. was required on direct gastric lavage. Thus a ratio of roughly 4:1 was established. These observations upon threshold dose were not supported by any detailed data or experimental protocols.

A series of sixty-three experiments were conducted to confirm these observations.

METHODS. Two dogs were prepared with vagotomized pouches of the entire stomach and esophago-jejunal anastomoses. The animals were fasted for ten to fourteen hours before each experiment. They were trained to stand in stocks while an experiment was in progress.

Basal secretion was collected for two and one-half hours and titrated against 0.0278 N NaOH(1 cc. equals 1 mgm. HCl) for free and total acid with Topfer's reagent and phenolphthalein. If any free acid was present during this period, the animal was not used for experimentation at that time.

The intravenous injections extended over exactly thirty minutes. They were given by slow continuous drip into the basilic vein. The gastric secretion, during the thirty minute injection period and for two hours following, was collected and titrated in the same manner as the basal secretion.

Direct gastric lavage was performed according to the method of Shoch and Bucher (7). This method consists of the insertion of a small rubber catheter

into the stomach pouch.   The catheter is connected to one outlet of a three-way stopcock.   A second outlet of the stopcock is connected to a piece of flexible rubber tubing draining into a small beaker which is suspended directly under the stoma of the pouch.   The third outlet of the stopcock is attached to a 50 cc. syringe.   This method permits the solution to be perfused into the pouch and the overflow allowed to drain back into the beaker by the proper adjustment of the stopcock.   The overflow is then aspirated into the syringe (by turning the stopcock) and reperfused into the pouch (by again turning the stopcock).   This procedure is continued for the thirty minutes of the lavage period.

At the end of the thirty minute period, the perfusate is allowed to drain into the beaker as completely as possible, and is emptied into a graduated cylinder.

A large rubber catheter, with numerous side openings to allow for drainage, is then inserted into the pouch in place of the smaller catheter.   Fifty cubic centimeters of distilled water is then perfused into the pouch and allowed to drain out immediately into the beaker.   This washing is combined with the perfusate and the volume measured.

Post-lavage secretion is collected for two hours and titrated in the same manner as for intravenous administration.

It is not possible to use accurately direct colorimetric titration for the lavage solution due to: 1, the color of the solutions; 2, the fact that the extract solution before lavage is not at neutrality; 3, the possible buffering action of the various constituents of the solution.

The pH of a sample of the original extract solution is determined by use of the glass electrode potentiometer.   The pH of the lavage solution is determined and the solution titrated back to its original pH value with 0.0278 N NaOH (1 cc. equals 1 mgm. HCl).

This method of estimating acid output has been found reliable by studies with solutions of known acid content.

The liver extract used in this series of experiments was from a single batch of Lederle's Liver Concentrate, 15 Anti-Pernicious-Anemia units per cc.[1]   The extract contained no phenol or preservatives, and was filtered through a large Berkefeld filter and stored on ice in large sealed serum bottles.   This extract was found to contain no vasodilatins by blood pressure studies on anesthetized dogs and action upon isolated guinea-pig intestinal strips.

Measured amounts of the extract were diluted to 50 cc. with normal saline immediately before use.   The saline was prepared from freshly distilled water before each experiment.   The solution was then filtered through two thicknesses of No. 500 filter paper.

Graduated amounts of the extract were used so that the minimal stimulating dose could be determined.   The secretory response, in terms of total acid output, caused by different dosages of the liver extract is presented in the accompanying table.

RESULTS.   It is evident from the data shown in table 1 that secretagogues applied directly to the stomach are more potent than secretagogues injected

[1] The liver extracts were generously provided by Dr. Y. Subbarow of Lederle, Inc.

intravenously. In dog 4, the lavage threshold dose is approximately 2.0 cc. of extract, while the intravenous threshold dose lies between 10 cc. and 20 cc. of extract. In dog 6, the lavage threshold dose is about 2.0 cc. of extract while the intravenous threshold dose is 5.0 cc.

TABLE 1

Total acid

GASTRIC LAVAGE				INTRAVENOUS		
Dose extract cc.	Total acid Mgm. HCl	Trials		Dose extract cc.	Total acid mgm. HCl	Trials
Dog 4						
2.0	0.1	2		5.0	1.8	3
3.0				10.0	1.2	3
4.0	6.8	1		20.0	0.9	2
5.0	5.8	3		30.0	5.2	2
6.0	27.5	5		40.0	43.9	2
7.0	14.4	3				
8.0	5.1	3				
9.0						
10.0						
11.0	22.6	1				
12.0						
13.0						
14.0	17.0	1				
Total trials..................		19				12
Dog 6						
2.0	1.0	2		5.0	0.0	1
3.0	13.5	2		10.0	3.8	3
4.0	17.0	3		20.0	10.5	2
5.0	9.7	2		30.0	21.8	2
6.0	27.3	3		40.0	14.7	2
7.0	34.7	3				
8.0	36.4	3				
9.0	39.9	1				
10.0						
11.0	41.9	2				
12.0						
13.0						
14.0	46.6	1				
Total trials..................		22				10

The reported observation of a ratio of 4:1 for threshold doses (6) is not confirmed by these data; the ratio is subject to variation between dogs. However, the more important observation of Kim and Ivy (6) is confirmed, namely, that secretagogues are more potent when applied to the gastric mucosa than when injected intravenously.

DISCUSSION. A humoral mechanism of gastric secretion has been established (2, 3, 4, 5). This humoral mechanism for the gastric phase of gastric secretion involves one of three general processes: 1, gastric secretatogues, present in foods, pass directly through the gastric mucosa and pass via the blood and lymph channels to the gastric secretory mechanism; 2, gastric secretagogues, present in foods, become altered in the stomach wall and then pass into the blood and lymph in an altered form to reach the gastric secretory mechanism; 3, gastric secretagogues, present in foods, stimulate the formation of a new substance, a hormone, in the gastric mucosa or submucosa which passes into the blood and lymph and stimulates the gastric secretory mechanism.

CONCLUSION

The results of this study indicate that secretagogues do not act by passing directly into the blood in an unchanged form, but act either in an altered form, the alteration being due to gastric juice, or by stimulating the elaboration of a gastric hormone.

REFERENCES

(1) EDKINS, J. S. J. Physiol. 34: 133, 1906.
(2) IVY, A. C. AND J. I. FARRELL. This Journal 74: 639, 1925.
(3) KLEIN, E. AND H. ARNHEIM. Arch. Surg. 25: 433, 1932.
(4) LIM, R. K. S., C. T. LOO AND A. C. LIU. Chinese J. Physiol. 1: 51, 1927.
(5) GREGORY, R. A. AND A. C. IVY. Quart. J. Exper. Physiol. 31: 111, 1941.
(6) KIM, M. S. AND A. C. IVY. This Journal 105: 220, 1933.
(7) SHOCH, D. AND G. BUCHER. Unpublished data.

STUDIES ON THE EFFECT OF THYMOXYETHYLDIETHYLAMINE (929 F) AND N-DIETHYLAMINOETHYL-N-ETHYLANILINE ON GASTRIC SECRETION IN THE DOG[1]

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Bovet and Staub (1937) reported that thymoxyethyldiethylamine (929 F) antagonized some of the physiologic actions of histamine. Staub (1939) found that N-diethylaminoethyl-N-ethylaniline (1571 F) was, in some of its actions, a more active antagonist of histamine than 929 F. Loew and Chickering (1941) found that 929 F did not decrease but significantly *increased* the gastric secretory response to histamine from Heidenhain pouches in dogs. Bourque and Loew (1943) reported that 1571 F reduced gastric secretion after stimulation by both histamine and pilocarpine, but failed to influence the gastric secretory response to a meal from dogs that had Heidenhain pouches. Burchell and Varco (1942) found that neither 929 F nor 1571 F affected the secretory response of dogs that had Heidenhain pouches to histamine.

The present investigation was carried out to determine the effect of 929 F and 1571 F on the normal gastric secretory process by studying their action on the secretory response to a meal. The effects of 929 F on the gastric secretory response to mecholyl chloride and to small doses of histamine have also been studied.

METHODS. Gastric secretion was studied in ten dogs. Four had pouches of the Pavlov type (Gregory, Hallenbeck and Code, 1942), four had Heidenhain pouches and two had transplanted gastric pouches. The animals were fasted for eighteen to twenty-four hours before collections of gastric juice were begun.

Mecholyl chloride was administered at constant rates by the continuous intravenous drip method. Histamine diphosphate was administered both in dilute solution by the continuous intravenous drip method and by intramuscular injection of a mixture of histamine, beeswax and sesame oil, which resulted in slow absorption and prolonged action of the histamine (Code and Varco, 1942). Doses of histamine are expressed in terms of the base. 929 F and 1571 F were provided by the Abbot Laboratories. Dr. M. H. Power and Dr. H. L. Mason kindly analyzed these compounds for their chlorine and nitrogen content and determined their melting points. The 929 F was found to be pure. The 1571 F was found to be pure except for the presence of 8.1 per cent of water by weight. Doses of 929 F are expressed as the monohydrochloride; doses of 1571 F are expressed as the dihydrochloride.

Doses of 929 F varied from 1 to 4 mgm. per kilogram of body weight per hour

when the drug was given intravenously by continuous drip, and from 2 to 4 mgm. per kilogram per hour when it was given subcutaneously. These doses of the drug were subemetic. Slightly larger doses produced emesis. 1571 F was given by continuous intravenous drip in doses of 15 to 20 mgm. per kilogram per hour. With these and larger doses the drug was not emetic when given intravenously. The larger doses, however, produced a state of increased irritability suggestive of stimulation of the central nervous system. Neither drug caused elevation of body temperature.

In experiments in which the gastric secretory response to a meal was studied, the secretory stimulant consisted of raw lean horse meat. The weight of meat fed was constant for each dog throughout the study, ranging from 100 to 300 grams for the different dogs.

Samples of gastric juice were collected at thirty-minute intervals. When it had been established that fasting secretion was not present in amounts exceeding a fraction of a cubic centimeter, the secretory stimulus was given. The volume of each sample was determined and aliquots were titrated for free acid, Töpfer's reagent being used as the indicator.

RESULTS.   1. *The effect of 929 F on the gastric secretory response to histamine.* In tests on two dogs that had Heidenhain pouches, gastric secretion stimulated by histamine given by continuous intravenous drip at a dose of 150 micrograms per hour was not inhibited by the subcutaneous injection of 929 F. Indeed, in the majority of the tests the output of gastric juice was greater after administration of the 929 F than before its administration. A similar result was obtained when the histamine was given intramuscularly in a mixture with beeswax and sesame oil. These results are in agreement with the earlier findings of Loew and Chickering (1941).

2. *The effect of 929 F on the response of Heidenhain pouches to mecholyl chloride.* As Gray and Ivy (1937) have shown, there exists for a given dog a dose of mecholyl chloride which is optimal for stimulation of gastric secretion. Such optimal doses of the drug, given intravenously by continuous drip, were established for each of four dogs that had Heidenhain pouches. The volume and free acidity of the gastric juice secreted during the first two hours of the response to mecholyl chloride were compared with those of the juice secreted during the first two hours of the response to a mixture of mecholyl chloride and 929 F. In the latter case the 929 F was first given alone in physiologic saline solution for thirty minutes, and then the mecholyl chloride was added to the solution and the two were given simultaneously.

Twenty-six tests were conducted on the four dogs that had Heidenhain pouches. Three of the four dogs showed diminished output of juice and hydrochloric acid, and one of the dogs showed an increased output of juice when 929 F was given (table 1). The differences between the weighted mean values for the four dogs were 1.7 per cent for volume of juice and 8.5 per cent for output of hydrochloric acid, both figures indicating a decrease of secretion after administration of 929 F. However, the standard errors of the various mean differences were large. In no case was a mean difference as much as three times the magni-

tude of its standard error, the criterion here accepted to indicate significance of the figure. Thus, although three of the four dogs showed diminished volume and output of acid with 929 F, the magnitude of this reduction was definitely within the limits of the control observations. It has been concluded, therefore, that 929 F does not have any significant effect on the gastric secretory response to mecholyl chloride from Heidenhain pouches in dogs.

3. *The effect of 929 F and 1571 F on the gastric secretory response to a meat meal.* Secretory responses to a meal were studied in four dogs that had Pavlov pouches, two dogs that had Heidenhain pouches and two dogs that had transplanted gastric pouches. During some of the control trials in all of the animals physiologic saline solution was given intravenously by the continuous drip method at a rate of 50 cc. per hour for the first one or one and a half hours after the meal was fed. This procedure duplicated the conditions of the trials in which either 929

TABLE 1

The effect of 929 F on the first two hours of the gastric secretory response to mecholyl chloride of four dogs that had Heidenhain pouches

DOG	EXPERIMENTS		DIFFERENCE BETWEEN SECRETION WITHOUT 929 F AND THAT WITH 929 F			
	Without 929 F	With 929 F	In volume of secretion		In output of hydrochloric acid	
			Mean difference	Per cent	Mean difference	Per cent
			cc.		*mgm.*	
1	3	3	−0.3 ± 2.5*	−3.3	−7.3 ± 8.0	−33.3
2	3	3	−4.6 ± 3.6	−34.6	−25.4 ± 20.5	−48.7
3	4	4	+4.0 ± 11.2	+12.0	+24.5 ± 67.5	+15.4
4	3	3	−1.9 ± 3.5	−11.3	−27.4 ± 16.8	−41.1
Total....	13	13	−0.3 ± 3.2	−1.7	−6.3 ± 18.2	−8.5

* Standard error.

F or 1571 F was used. In no case did administration of physiologic saline solution alone inhibit the gastric secretory response to the meat meal.

(a) *Pavlov pouches.* 929 F and 1571 F inhibited the gastric secretory response to a meal in dogs that had Pavlov pouches. In general, such inhibition became evident about thirty minutes after the administration of either of the drugs had been started. Maximal inhibition usually persisted for a half to one hour after the administration of either of the drugs had been stopped. During the following two hours, the rate of secretion gradually increased. During the first two hours after ingestion of the meal the inhibition of volume of juice and output of hydrochloric acid after administration of 929 F varied from about 30 per cent to about 80 per cent of the control values, while that for 1571 F varied from about 65 per cent to 90 per cent of the control values (tables 2 and 3). The differences were found to be statistically significant.

(b) *Heidenhain pouches.* One dog (Heidenhain 1, table 4) received 929 F subcutaneously in a dose of 2 mgm. per kilogram of body weight at the time of feeding. The volume of juice and output of hydrochloric acid were reduced

TABLE 2

The effect of 929 F on the first two hours of the gastric secretory response of pouches of the Pavlov type to a meal meal

DOG	EXPERIMENTS		DIFFERENCE BETWEEN SECRETION WITHOUT 929 F AND THAT WITH 929 F			
	Without 929 F	With 929 F	Reduction of volume of secretion		Reduction of output of hydrochloric acid	
			Mean difference	Per cent	Mean difference	Per cent
			cc.		*mgm.*	
1	7	4	35.0 ± 5.0*	71.6	194.6 ± 25.3	78.5
2	12	3	27.6 ± 4.4	46.2	157.8 ± 28.6	47.9
3	6	4	18.5 ± 4.7	68.3	101.7 ± 26.0	74.1
4	7	4	19.4 ± 4.5	31.6	106.8 ± 25.6	32.6
Total....	32	15	28.0 ± 1.8	54.3	158.2 ± 13.2	57.5

* Standard error.

TABLE 3

The effect of 1571 F on the first two hours of the gastric secretory response of pouches of the Pavlov type to a meat meal

DOG	EXPERIMENTS		DIFFERENCE BETWEEN SECRETION WITHOUT 1571 F AND THAT WITH 1571 F			
	Without 1571 F	With 1571 F	Reduction of volume of secretion		Reduction of output of hydrochloric acid	
			Mean difference	Per cent	Mean difference	Per cent
			cc.		*mgm.*	
1	7	2	36.3 ± 3.8*	74.2	197.2 ± 20.1	79.5
2	12	2	39.0 ± 2.6	65.3	234.9 ± 17.8	71.4
3	6	2	21.9 ± 4.6	80.8	122.9 ± 25.9	90.0
4	7	2	43.3 ± 4.3	70.5	245.8 ± 32.1	74.9
Total....	32	8	37.4 ± 3.7	72.5	214.7 ± 20.5	78.0

* Standard error.

TABLE 4

The effect of 929 F on the first two hours of the gastric secretory response of Heidenhain and transplanted gastric pouches to a meat meal

DOG	EXPERIMENTS		REDUCTION OF VOLUME OF SECRETION		REDUCTION OF OUTPUT OF HYDROCHLORIC ACID	
	Without 929 F	With 929 F	Mean difference	Per cent	Mean difference	Per cent
			cc.		*mgm.*	
Heidenhain 1..........	4	3	6.2 ± 1.8*	36.7	31.5 ± 14.8	45.9
Heidenhain 2..........	4	3	8.9 ± 1.7	65.4	49.9 ± 10.1	83.0
Transplant 1..........	3	1	4.6	59.0	11.9	100.0
Transplant 2..........	4	4	11.6 ± 0.5	80.6	60.5 ± 1.5	89.1

* Standard error.

36.7 and 45.9 per cent respectively. The other dog (Heidenhain 2, table 4) received 929 F by continuous intravenous drip for one hour after feeding. The mean inhibition of the volume of juice secreted during the first two hours was 65.4 per cent; that of the output of hydrochloric acid was 83.0 per cent. With one exception, all mean differences were statistically significant.

(c) *Transplanted gastric pouches.* One dog (transplant 1, table 4) was given multiple subcutaneous injections of 929 F at intervals after ingestion of the meal. No free hydrochloric acid was secreted during the first two hours, a phenomenon which did not occur in three trials without the drug. The other dog (transplant 2, table 4) received 929 F by continuous intravenous drip for one hour after feeding. The volume of juice and the output of hydrochloric acid were inhibited 80.6 and 89.1 per cent respectively when 929 F was given. The mean differences were statistically significant.

Thus, throughout these experiments 929 F uniformly inhibited the secretory response of Pavlov, Heidenhain and transplanted gastric pouches to the ingestion of a meat meal. Likewise, 1571 F inhibited the secretory response of Pavlov pouches to the ingestion of a meat meal. The action of 1571 F on the response of Heidenhain and transplanted gastric pouches to a meat meal was not tested.

One dog that had a transplanted gastric pouch was prepared with a Mann-Bollman fistula of the stomach so that simultaneous records of gastric secretion and motility could be obtained. The secretory response of the pouch was inhibited as much as 80 per cent by 929 F without inhibition of gastric motility. This observation plus the finding in another study that inhibition of gastric motility by 929 F is inconstant (Hallenbeck, Code and Mann) indicates that inhibition of gastric motility plays a minor rôle, if any, in the inhibitory action of 929 F on the gastric secretory response to a meal.

COMMENT. The results after administration of 1571 F indicate that this drug inhibits the gastric secretory response of Pavlov pouches to the ingestion of a meat meal. Bourque and Loew (1943), however, observed that 1571 F failed to influence the response of Heidenhain pouches to a meal. The difference in the results is probably due to the difference in the nerve supply of the two types of pouches.

There is a possibility that part of the inhibition exercised by 929 F on the response of Pavlov pouch dogs to a meal may be the result of depression of psychic stimulation by the nausea which the drug may cause. Atkinson and Ivy (1938) have shown that apomorphine, emetine hydrochloride and quinine in emetic and subemetic doses can inhibit the gastric secretory response to histamine in dogs that have Pavlov pouches. However, the fact that the response to a meal is also inhibited in Heidenhain and transplanted pouch preparations in dogs treated with 929 F shows that inhibitory stimuli or diminution of psychic secretory stimuli reaching the gastric glands of the pouches via nerves is not an all-important factor.

The data in this report do not suggest that the inhibition of the gastric secretory response to a meal in dogs by 929 F and 1571 F is the result of direct antagonism of these drugs to histamine.

SUMMARY AND CONCLUSIONS

A study was made of the effect of 929 F and 1571 F on the secretory response of gastric pouches in dogs.   The results were as follows:

1. The finding of other investigators that 929 F does not inhibit the secretory response of Heidenhain pouches to histamine was confirmed.

2. 929 F did not significantly alter the gastric secretory response of Heidenhain pouches to mecholyl chloride.

3. 929 F inhibited the gastric secretory response of Pavlov, Heidenhain and transplanted pouches to the ingestion of a meat meal.

4. 1571 F inhibited the gastric secretory response of Pavlov pouches to the ingestion of a meal.

I should like to express my gratitude to Dr. Charles F. Code for his help and guidance throughout the course of this study.

REFERENCES

ATKINSON, A. J. AND A. C. IVY.   Am. J. Digest. Dis. and Nutrition 4: 811, 1938.
BOURQUE, J. E. AND E. R. LOEW.   This Journal 138: 341, 1943.
BOVET, D. AND A.-M. STAUB.   Compt. Rend. Soc. de biol. 124: 547, 1937.
BURCHELL, H. D. AND R. L. VARCO.   J. Pharmacol. and Exper. Therap. 75: 1, 1942.
CODE, C. F. AND R. VARCO.   This Journal 137: 225, 1942.
GRAY, J. S. AND A. C. IVY.   This Journal 120: 705, 1937.
GREGORY, R. A., G. A. HALLENBECK AND C. F. CODE.   Proc. Soc. Exper. Biol. and Med. 49: 400, 1942.
HALLENBECK, G. A., C. F. CODE AND F. C. MANN.   Gastroenterology (In press).
LOEW, E. R. AND O. CHICKERING.   Proc. Soc. Exper. Biol. and Med. 48: 65, 1941.
STAUB, A.-M.   Ann. Inst. Pasteur. 63: 400, 485, 1939.

THE EFFECTS OF HYPERVENTILATION AND OF BLOOD-PRESSURE CHANGES ON THE SELF-SUSTAINED RESPONSES OF THE CEREBRAL CORTEX

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The marked influence of hyperventilation on the spontaneous activity of the cerebral cortex has been repeatedly studied and over-breathing tests have become a routine procedure in clinical electro-encephalography. Nevertheless, very little is known about the action of CO_2 changes on the provoked cortical responses. Information on this point might bring some light on several obscure points.

Brody and Dusser de Barenne (1932) studied the effect of hyperventilation on the responses to electrical stimulation of the cortex by recording the muscular contraction provoked by the stimuli. With this procedure, however, several structures intervene between the cortex and the reacting muscles, that might react in different ways to the hyperventilation.

As opposed to this indirect method of approach, the study of the electrical self-sustained "tonic-clonic" responses recently analyzed by Rosenblueth and Cannon (1942) gives direct evidence about some cortical properties and their variations under different experimental conditions.

METHOD. Cats were used. In most of the experiments dial (Ciba, 0.65 cc. per kgm. intraperitoneally) was the anesthetic. Some animals were studied after a mesencephalic transection (Bremer, 1935a); in these cats a short period of ether anesthesia preceded the transection; bleeding during the operation was prevented by intermittent occlusion of the carotid arteries.

Tracheal cannulae with a side hole were used. Hyperventilation was induced by increasing the amount of air delivered or by speeding up the frequency of an artificial-respiration pump. A double action (suction and thrust) pump was used in order to give definite gas mixtures contained in Tissot gasometers.

The blood pressure was recorded in most of the experiments from the left carotid artery by means of a Hürthle manometer. Four per cent sodium citrate solution was employed for prevention of clotting and care was taken to prevent the animal receiving appreciable amounts of that solution during falls of blood pressure, since a large and sometimes irreversible decrease of cortical activity was observed when this occurred accidentally.

The cerebral hemispheres were exposed and the stimulating and recording electrodes (silver wires with a small bead at the tip) were applied to the surface of the pia. The interelectrodal distance was about 5 mm. both for stimulation and for recording. The stimuli were series of induced shocks from a Harvard coil with 3 volts on the primary. The maximal distance between the coils that

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yielded a well-developed "self-sustained response" was used.   This distance was about 8 cm. in most cases.   The duration of the trains was ordinarily 2 to 3 seconds and a constant interval of 2 to 3 minutes was allowed between successive periods of stimulation.   Possible interfering facilitation and extinction factors (see Dusser de Barenne and McCulloch, 1939) were thus minimized.

The electrical phenomena in the cortex were recorded from 2 to 6 pairs of silver electrodes applied to the pia on different regions in one or both cerebral hemispheres.   After capacity-coupled amplification the responses were recorded by means of ink-writing moving-coil galvanometers (six-channel Grass oscillograph).   The amplifiers were operated in push-pull.   The time constant was 0.15 second.   The animal was grounded through a lead attached to the head-holder.

In the experiments in which occlusion of the abdominal aorta was performed a thread was passed under the artery above the renal arteries and both ends of the thread were then inserted into a piece of glass tubing; a hemostat supporting the thread on the free end of the tubing permitted an adequate regulation of the degree of occlusion.   This device allowed closure of the abdominal cavity during the experiments.

RESULTS.   A. *The self-sustained responses during hyperventilation.*   We call "self-sustained responses" the periods of electrical events recorded from the cortex after adequate cortical stimulation.   By extension of the term applied to the muscular contractions that under certain conditions accompany such cortical responses, these will also be called "tonic-clonic" (Rosenblueth and Cannon, 1942).

Hyperventilation with ordinary air invariably produced a decrease and eventually a suppression of the self-sustained responses.   The time required for that effect was 1 to 5 minutes in most animals.   The decrease in the self-sustained responses occurred not only as a shortening of the period of activity but also as a fall in voltage and a slowing of rate of the cortical discharges.   In every instance either or both of these changes were evident.   Thus the total energy consistently decreased (fig. 1).   Increases were not observed.

The changes in cortical activity were reversible, provided the periods of hyperventilation were not too long.   The recovery toward the original normal response was progressively slower after longer hyperventilation.   Multiple leads from both cerebral hemispheres were used in order to study the influence of hyperventilation on the spread of the tonic-clonic responses.   The changes were more or less parallel in all the leads (fig. 2).

A change of the type of the self-sustained responses to the cortical stimuli was sometimes observed during hyperventilation.   The initial fast components decreased first and the clonic stage seemed to be relatively prolonged, although the total duration of the response was reduced.

The changes described were present when hyperventilation was tried after bilateral section of the vagi.   Cats with a mesencephalic transection showed also similar changes and the same phenomenon was observed in animals in which blood-pressure changes were prevented, as will be described under C.

B. *The self-sustained responses with impaired cerebral circulation.* Blood-pressure records showed a marked fall during hyperventilation; an immediate
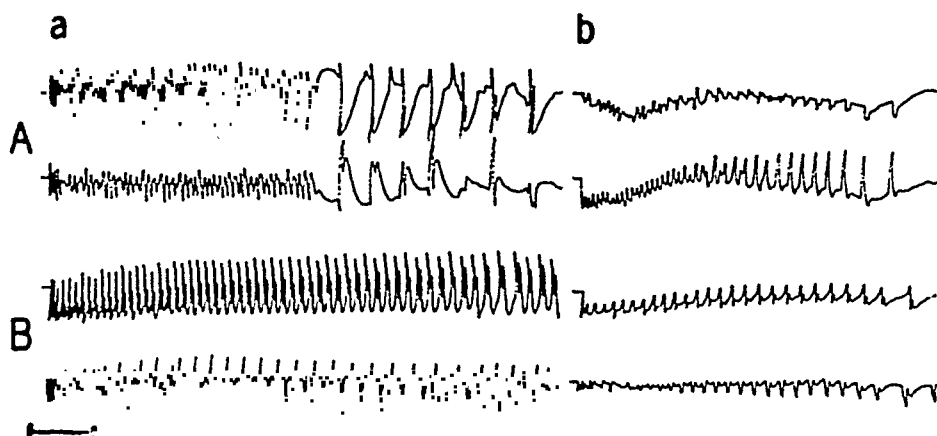
Fig. 1. Decrease of the self-sustained response of the cerebral cortex of the cat, after hyperventilation with ordinary air. The records begin shortly after the stimulus had been applied. This and the following electrical records were taken with capacity-coupled amplifiers and ink-writing galvanometers. Time calibration: 0.5 sec.

A. Responses to faradic stimulation (2 sec.), before, *a*, and after, *b*, 2 min. of hyperventilation.

B. The fall of blood pressure was prevented by compressing the abdominal aorta. Responses to the same faradic stimulations before, *a*, and after, *b*, 4 min. of hyperventilation.
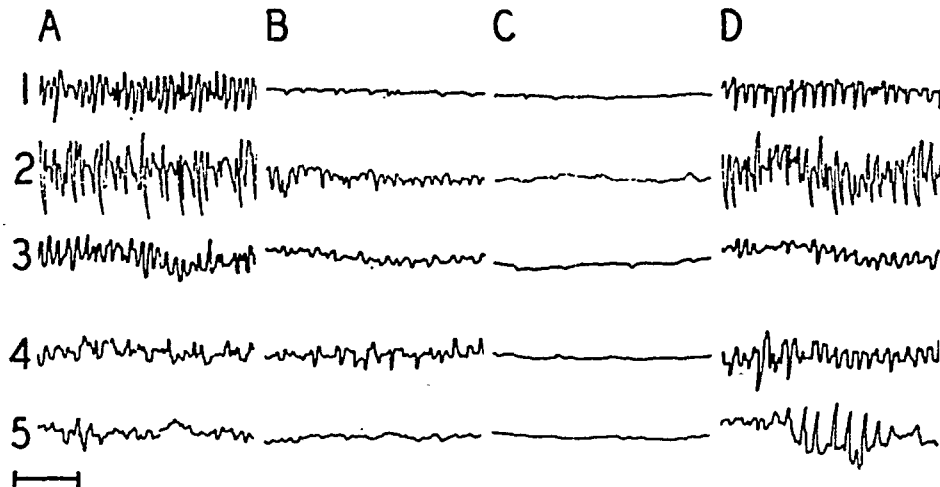
Fig. 2. Simultaneous effects of hyperventilation at several regions of the cerebral cortex. Stimulating electrodes on left suprasylvian gyrus, middle portion. Records 1, 2 and 3 from pairs of electrodes on the frontal, temporal and occipital regions of the left hemisphere respectively; 4 and 5 from the frontal and posterior parts of the right suprasylvian gyrus. A to D are strips showing the responses about 2 sec. after their beginning. Calibration: 0.5 sec.

A, normal response. B and C, after 3 and 5 min. of hyperventilation, respectively. D, 6 min. after the hyperventilation was stopped.

drop was followed after some irregular waves by a secondary slow progressive decrease. This striking change of blood pressure required some controls.

First the effect on the self-sustained responses of some other procedures that produce a decrease of blood pressure was tested, i.e., electrical stimulation of the peripheral ends of the vagus nerves (fig. 3 A) and obstruction of the venous return by compression of the heart (fig. 3 B). Under both of these conditions the cortical responses were markedly decreased.

In some demonstrative experiments after the self-sustained responses were clearly decreased by hyperventilation, a definite improvement was obtained by bringing the blood pressure back to normal by the procedure described in C; they became depressed again when the pressure was released.

The obvious mechanism suggested by these results was that the depression was caused by the ischemic condition of the brain. This factor was tested by clamping the intact carotid (the other one being previously occluded for blood
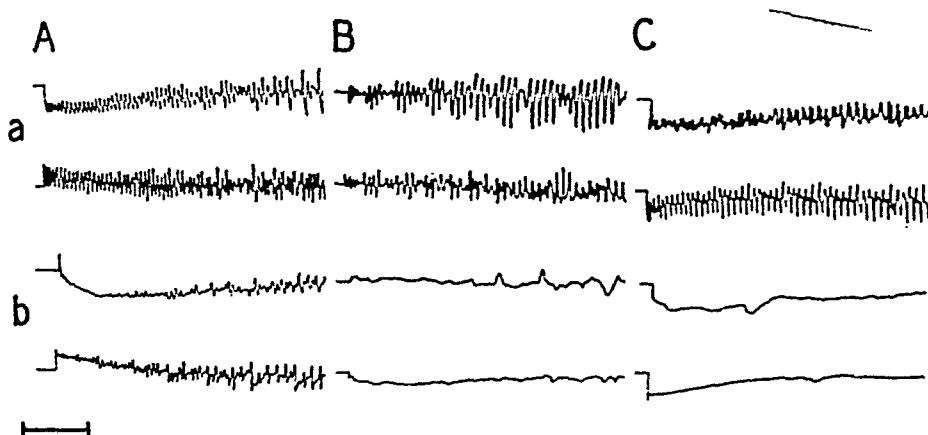
Fig. 3. Effect of decreased blood pressure and impaired cerebral circulation on the self-sustained responses of the cerebral cortex. The figure shows in each case the beginning of the response to cortical stimulation. Time calibration: 0.5 sec.

A. Before, a, and after, b, 40 sec. of stimulation of the peripheral end of the left vagus.

B. Before, a, and after, b, 1 min. of obstruction of the venous return by compression of the heart.

C. Before a, and after, b, 1 min. of occlusion of both carotid arteries.

pressure recording). The cortical responses were quickly reduced in spite of the reflex increase of blood pressure. Half a minute after the clamping, the responses were already reduced; 1 to 3 minutes of occlusion were enough to suppress any cortical response and even the spontaneous activity disappeared (fig. 3 C).

C. *Hyperventilation without changes of blood pressure.* In order to eliminate the circulatory factors, the fall of blood pressure that occurs during hyperventilation was avoided by simultaneous occlusion of the abdominal aorta. The marked increase of pressure that can be obtained by this procedure was adjusted to the desired value, as described under Methods (p. 335). Despite the unchanged blood pressure, acapnia resulting from hyperventilation decreased the cortical responses, but the effect took a longer time to become evident and it was less marked than the decrease when the blood pressure went down (fig. 1 B). Increases of blood pressure above normal values during normal breathing failed to produce any change of the responses.

D. *Hyperventilation without acapnia.* Hyperventilation without acapnia was obtained by the administration of air with 4 to 5 per cent CO_2. There was no decrease of the self-sustained responses if the blood pressure was kept normal, but some decrease when the blood pressure was not prevented from falling (fig. 4).

Hyperventilation with high concentrations of CO_2 (8 to 10 per cent) did not decrease cortical responses. Clamping of the aorta was then unnecessary to preserve a normal blood pressure, since after an initial and brief drop, the pressure recovered by itself to its previous value (fig. 4 C).

E. *The spontaneous activity of the cortex.* It was not the purpose of this study to consider systematically the changes of spontaneous activity during hyperventilation, and only incidental observations will be reported here. In general
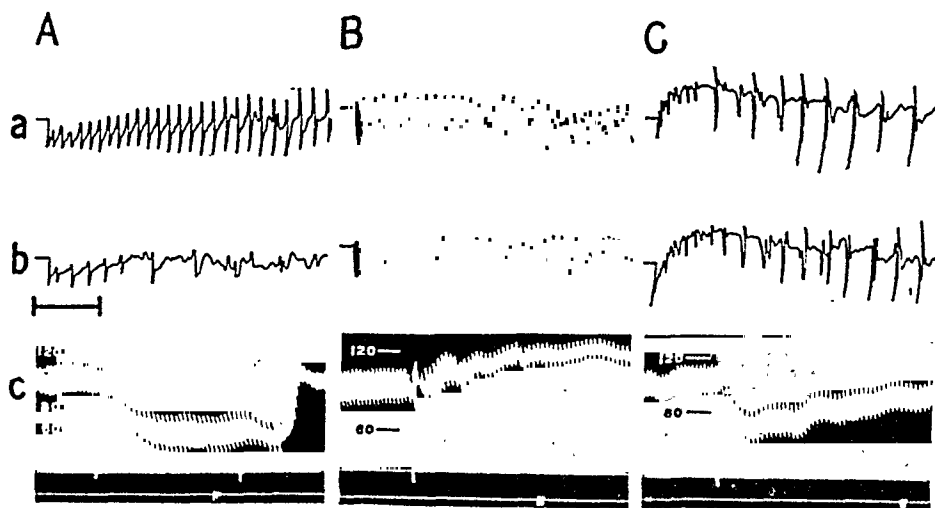
Fig. 4. Effect of hyperventilation without acapnia on the self-sustained response of the cerebral cortex. The electrical records show the beginning of the responses to cortical stimuli. The kymographic records show the blood-pressure changes during the periods of hyperventilation, as indicated by the upper signal. The lower signal marks cortical stimulations. Calibrations, for *a* and *b*: 0.5 sec.; for *c*: millimeters of mercury.

A. Before, *a*, and after, *b*, 1 min. of hyperventilation with 4 per cent CO_2.
B. As in A, but the fall of blood pressure was prevented by occlusion of the aorta. The record of Bb was taken after 5 min. of hyperventilation.
C. Before, *a*, and after, *b*, $3\frac{1}{2}$ min. of hyperventilation with 8 per cent CO_2.

it may be said that the spontaneous activity presented changes in the same direction as those of the self-sustained responses. In other words, hyperventilation produced always a decrease of activity (fig. 5); this decrease was more striking when a fall of blood pressure accompanied the acapnia, but it could be seen also when that fall was prevented. This effect was found also in cats with mesencephalic transection in opposition to what Bremer (1935b) reported. There was no temporal correlation, however, between the changes in spontaneous activity and those in the tonic-clonic responses. Thus, the decrease of the latter responses occurred sometimes before any change of the spontaneous background was detectable; and sometimes the background spontaneous waves decreased first. We are unable to report the changes in pattern of the background activity, because they were not systematic in the short records taken.

By "decrease of activity" is meant that in general there were simultaneous falls of voltage and frequency which predominated over any occasional increase in the duration of some potential changes. Frequently the peculiar pattern in the cortical activity of an animal under barbituric anesthesia was maintained throughout an experiment and variations were observed only in the voltage and frequency of the individual waves of the "spindles."

DISCUSSION. Hyperventilation decreases the self-sustained responses of the cerebral cortex. This effect was invariably observed in these experiments, as reported in section A. The decrease affected three features of the responses: the duration, and the voltage and frequency of the components. It is interesting to contrast these results with the ability of hyperventilation to induce attacks of "petit mal" in epileptic patients (Foerster, 1924).

The report of Brody and Dusser de Barenne (1932) that hyperventilation causes an increase of the motor responses to cortical stimulation seems to be in contradiction with the present observation. It is pertinent to point out, how-
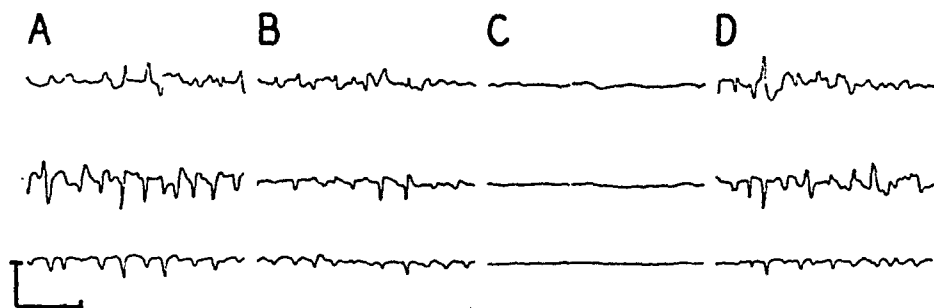
Fig. 5. Effects of hyperventilation on the spontaneous activity of the cerebral cortex of the cat. Electrodes on the frontal, middle and posterior portions of the suprasylvian gyrus. Calibrations: 0.5 sec. and 500 μv.

A. Normal record. B and C, after 1 and 3 min. of hyperventilation, respectively. D, 1½ min. after the hyperventilation was stopped.

ever, that Brody and Dusser de Barenne studied responses which were not the electrical potentials of the cortex but muscular contractions recorded by a Marey tambour. It is evident that in that procedure there are many steps and structures involved that may react to hyperventilation in different or even opposite ways. Striking among such possible effects is the increase of excitability of nerve and the change of accommodation that sometimes make "repetitive" the responses to single-threshold shocks (Rosenblueth and del Pozo, 1942). Brody and Dusser de Barenne reported also in the same paper some observations under strychnine in which hyperventilation or $NaHCO_3$ not only produced bigger responses but also a shortening of latency, epileptoid after-discharges, and spread to other muscular groups. These effects suggest a central action of hyperventilation, but the number of factors at play under those conditions makes an evaluation difficult.

Variable effects of hyperventilation on different structures may explain why, notwithstanding the remarkable action that acapnia has on the excitability and accommodation of nerve, a reflex may be decreased, as was the knee-jerk in the studies of Henderson (1910), or may be unchanged, as in four cats tested in our

series for the effect of hyperventilation on the flexor reflex. Decrease of hearing (Gellhorn and Spiesman, 1935), of visual intensity discrimination (Gellhorn, 1936) and several other depressant actions may be mentioned. The increase of visual sensitivity of the dark-adapted eye (Wald, Krieger and Goodman, 1942) further illustrates the variability of the effects of hyperventilation, depending on the structure considered.

The marked fall of blood pressure during hyperventilation showed an initial drop and a secondary progressive decrease. When the hyperventilation was carried out with gaseous mixtures containing over 4 per cent CO_2, the sharp initial drop persisted, but it was followed by a gradual recovery of the previous normal blood pressure. These results demonstrate that only the initial fall of blood pressure is due to the change in the intrathoracic pressure. The progressive secondary fall that takes place when ordinary air is used for hyperventilation is due to the acapnia induced (Dale and Evans, 1922).

Striking decreases both in self-sustained cortical responses and in spontaneous activity were observed in our experiments during decreases of blood pressure induced without changes in respiration (p. 338, fig. 3). Beecher, McDonough and Forbes (1938) found the same effect on spontaneous activity during falls of blood pressure produced by Cannon's method of cardiac tamponade (Cannon, 1923). Such remarkable decreases in cortical activity are probably due to cerebral ischemia (p. 338, fig. 3 C). The recovery from the ischemic effects was very slow in spite of the fact that the vertebral arteries were not occluded (see Stewart, Guthrie, Burns and Pike, 1906). Large as the effect of the fall of blood pressure is in decreasing the cortical activity, during hyperventilation the acapnia adds its independent effect, which was evident in the experiments under normal blood pressure (p. 338, fig. 1 B).

The spontaneous activity was not specially studied, but our incidental observations are in accord with other reports on the slow frequency of the components during hyperventilation (p. 339, fig. 5). Gibbs, Williams and Gibbs (1940), using the Grass analyzer, found that associated with low CO_2 tension in the jugular blood during hyperventilation there is an increase in the total energy and a shift toward the slow end of the frequency spectrum in the electroencephalogram. We did not notice any obvious increase of energy, and the occasional changes of pattern were not consistent enough to warrant detailed discussion.

It would be natural to suppose that the hyperventilation that we produced would cause an acapnia, but we have no direct information about the state of the cortex. Dusser de Barenne, Marshall, McCulloch and Nims (1938) have found changes in the local pH of the cortex correlated with cortical activity which were not always parallel with the pH of the blood. Furthermore, the vasoconstrictor effect of a reduction of CO_2 may oppose the loss of CO_2 from the cortex. Gibbs, Gibbs, Lennox and Nims (1942) present evidence that indicates the primary importance of the CO_2 tension of the brain in determining the electroencephalographic changes that occur during and after hyperventilation. Davis and Wallace (1942) had previously suggested that the changes might be the result of anoxia produced by the vasoconstriction. Since in our experiments neither

CO_2 tensions nor O_2 tensions were measured, any discussion about the mechanism underlying the changes that we observed in the cortical activity would be purely speculative.

SUMMARY

The effect of hyperventilation on the self-sustained responses of the cortex was studied in cats. Hyperventilation produced a decrease of the self-sustained responses. This decrease affected the duration of the response, and the voltage and frequency of the discharges (p. 336, figs. 1 and 2).

The decrease of the responses was observed in animals in which the blood pressure was kept unchanged during hyperventilation (p. 338, fig. 1 B).

Induced falls of blood pressure, with normal respiration, produced also marked decrease of the self-sustained responses (p. 338, figs. 3 A and B). Cerebral ischemia by bilateral carotid occlusion resulted in the same effect (p. 338, fig. 3 C).

Incidental observations were made on the effects of hyperventilation on the spontaneous activity of the cortex. Decreases in voltage and frequency were found during hyperventilation (p. 339, fig. 5).

REFERENCES

BEECHER, H. K., F. K. McDONOUGH AND A. FORBES.   J. Neurophysiol. 1: 324, 1938.
BREMER, F.   Compt. rend. Soc. Biol. 118: 1235, 1935a.
          Ibid. 118: 1241, 1935b.
BRODY, B. S. AND J. G. DUSSER DE BARENNE.   Arch. Neurol. and Psychiat. 28: 571, 1932.
CANNON, W. B.   Traumatic shock.   New York, 1923, p. 63.
DALE, H. H. AND C. L. EVANS.   J. Physiol. 56: 125, 1922.
DAVIS, H. AND W. McL. WALLACE.   Arch. Neurol. and Psychiat. 47: 606, 1942.
DUSSER DE BARENNE, J. G., C. S. MARSHALL, W. S. McCULLOCH AND L. F. NIMS.   This Journal 124: 631, 1938.
DUSSER DE BARENNE, J. G. AND W. S. McCULLOCH.   J. Neurophysiol. 2: 319, 1939.
FOERSTER, O.   Deutsch. Ztschr. f. Nervenh. 83: 347, 1924.
GELLHORN, E.   This Journal 115: 679, 1936.
GELLHORN, E. AND J. G. SPIESMAN.   Ibid. 112: 519, 1935.
GIBBS, E. L., F. A. GIBBS, W. G. LENNOX AND L. F. NIMS.   Arch. Neurol. and Psychiat. 47: 879, 1942.
GIBBS, F. A., D. WILLIAMS AND E. L. GIBBS.   J. Neurophysiol. 3: 49, 1940.
HENDERSON, J.   This Journal 25: 310, 1910.
ROSENBLUETH, A. AND W. B. CANNON.   Ibid. 135: 690, 1942.
ROSENBLUETH, A. AND E. C. DEL POZO.   Ibid. 136: 629, 1942.
STEWART, G. N., C. C. GUTHRIE, R. L. BURNS AND F. H. PIKE.   J. Exper. Med. 8: 289, 1906.
WALD, G., H. P. KRIEGER AND H. C. GOODMAN.   Fed. Proc. 1: 88, 1942.

THE EFFECT OF CAFFEINE AND COFFEE EXTRACT ON THE ACTIVITY OF THE DIGESTIVE ENZYMES

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The results reported in the literature on the effect of coffee or its constituents on enzymic activity are meager. Sabalitschka and Schulze (1925) found caffeine to have no effect on malt amylase. Ohnishi (1928) reports activation of protease by caffeine. Heide and Schilf (1929) in experiments *in vivo* found that milk was more rapidly digested in the stomachs of rats when mixed with an infusion of coffee. Zlatarov and Popov (1937) conclude from the results of experiments in which the hydrogen ion concentrations of the substrates were not properly adjusted that caffeine has a slightly favorable influence on pepsin and trypsin but is without effect on lipase and ptyalin. In view of this lack of data, and because of the widespread belief that coffee interferes with digestion, it was thought that a careful and systematic investigation of the effect of caffeine and coffee extract on the activity of the digestive enzymes *in vitro* would be of value. The enzymes studied were those which act upon carbohydrates, proteins, and fats in the digestive tract, namely, ptyalin, pancreatic amylase, pepsin, trypsin, and pancreatic lipase.

EXPERIMENTAL PROCEDURES. · The water used in making up the substrates upon which the enzymes were allowed to act and all solutions added to them was obtained by distilling ordinary distilled water from alkaline potassium permanganate. This distillate was acidified with phosphoric acid and again distilled.

The coffee used was a commercial brand purchased as the roasted bean and ground. It was stored in a glass stoppered bottle. The extract was prepared by adding 5 grams to about 100 cc. of boiling water and allowing it to stand five minutes with occasional stirring. It was then filtered through a coarse filter paper and the filtrate made up to exactly 100 cc. and used within half an hour. The caffeine was Merck's U.S.P. powder further purified by recrystallization. In these experiments both the caffeine and coffee were tested in two concentrations, namely, 20 and 40 mgm. of caffeine and 20 and 40 cc. of coffee extract per 100 cc. of substrate.

In all the digestion mixtures the hydrogen ion concentration was most carefully checked and adjusted so that any variation in the action of the enzyme could not be attributed to this factor. The measurements were made electrometrically with a Leeds and Northrup Universal pH Indicator, a glass electrode being used.

Amylolytic digestions. The amyloclastic materials studied were ptyalin in fresh filtered saliva, and a high grade commercial pancreatin. Two cubic centimeters of the filtered saliva were made up to 50 cc. and 1.5 cc. of this diluted material was added to each digestion mixture. In the case of the pancreatin 1 cc. of a filtered solution containing 1 mgm. per cubic centimeter was used.

The activities of the salivary and pancreatic amylases were measured by determining the amount of reducing sugar formed. The method used was that of Sherman and co-workers (1921). The enzyme was allowed to act for 30 minutes at 40° C. on 100 cc. of a 1 per cent dispersion of soluble starch to which had been added as activators for the amylase 5 cc. of M sodium chloride and 2.5 cc. of 0.02 M disodium hydrogen phosphate. The pH of the substrate was in all tests adjusted to 7.0 by means of 0.01 M sodium hydroxide. At the end of half an hour the action of the enzyme was stopped by adding to the digestion flasks 50 cc. of Fehling's solution. These were immersed for 15 minutes in boiling water and the weight of cuprous oxide formed by the reducing sugar in this length of time was taken as a measure of the rate of digestion. Blanks were carried out under the same conditions but without added enzyme to determine the reducing action of the caffeine and of the coffee extract. In the case of the coffee extract the reducing action was found to be appreciable, so in all the results reported for coffee this value has been substracted. Caffeine showed no reducing action.

Proteolytic digestions. The protein splitting enzymes studied were high grade commercial pepsin and trypsin. One cubic centimeter of a 1 per cent solution of pepsin in 0.05 M hydrochloric acid was used for each digestion. The same amount of trypsin dissolved in water was used in each test with this enzyme. A substrate of 1 per cent casein (according to Hammarsten) was used. In the pepsin experiments 0.5 gram of casein was dispersed in 50 cc. of 0.05 M HCl, or in 50 cc. of the acid extract of coffee in the digestions in which the effect of coffee was studied, by warming to between 40° C. and 50° C. In the preparation of the caffeine solution and the coffee extract 0.05 M HCl was also used instead of water. The pH of the digestions mixtures was adjusted to 1.34. The pepsin was allowed to act on 50 cc. of the substrate for 1 hour at 40° at the end of which time the undigested casein was precipitated by the addition of 12.5 cc. of 20 per cent sodium sulphate. After standing overnight the mixture was filtered and the filtrate made up to 100 cc. The nitrogen in a 10 cc. portion of this filtrate was determined by a semimicro modification of the Kjeldahl method.

The same general procedure was carried out for trypsin digestions except that the casein was dispersed in a dilute sodium hydroxide solution. The pH of the substrate was 8.22. To the mixtures to which caffeine or coffee was added, sufficient sodium hydroxide solution was also added to adjust the pH to this same value.

Semimicro determinations of the nitrogen in the added caffeine and coffee extract were made, and this amount was substracted from the results obtained in the proteolytic digestions when these substances were present.

Lipolytic digestions. In these experiments the influence of caffeine and coffee on the digestion of olive oil by steapsin, the fat splitting enzyme secreted by the pancreas, was determined by the following procedure: 2 cc. of neutralized olive oil were measured into a 125 cc. Erlenmeyer flask. To this was added 1 cc. of 6 per cent bile salt (sodium taurocholate), 1 cc. of caffeine solution (or neutral coffee extract, or water in case of the control), and 1 cc. of steapsin solution. The flasks were shaken vigorously and placed in a bath at 40° C. for 2½ hours. At

frequent intervals during the digestion the shaking was repeated. At the end of the digestion period, 20 cc. of isopropyl alcohol were added and the amount of fatty acids formed was determined by titration with 0.05 M sodium hydroxide, bromthymol blue being used as indicator. Since all the digestion mixtures in a comparative set of experiments could not be titrated at the same time, it was necessary, in order to stop the enzymic action, to keep the flasks immersed in crushed ice during the interval between removal from the bath and titration.

In preliminary experiments carried out to determine the best conditions for the estimation of the fatty acids formed, it was found that a more satisfactory end point was obtained when isoprophyl alcohol was used instead of the ethyl alcohol usually employed in this determination. Because of the color of the coffee it was also found in the experiments with this material that phenolphthalein was worthless as an indicator, but that bromthymol blue gave a readily discernible and definite end point.

In the lipolytic experiments the volume of the substrate was 5 cc. so the amounts of caffeine added in the two series of experiments were 1 mgm. and 2

TABLE 1

Effect of coffee extract on salivary and pancreatic amylases and pancreatic lipase

COFFEE EXTRACT PER 100 CC. OF SUBSTRATE	SALIVARY AMYLASE		PANCREATIC AMYLASE		PANCREATIC LIPASE	
	Cu$_2$O		Cu$_2$O		0.05 M NaOH equiv. to acids formed	
cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*cc.*	*cc.*
None	296		246		23.1	
20	318		273		20.7	
None		339		195		21.3
40		353		218		13.2

mgm. which would be equivalent to 20 mgm. and 40 mgm. per 100 cc., the same concentrations used in the amylolytic and proteolytic digestions. Likewise the volumes of coffee extract added were equivalent to 20 cc. and 40 cc. per 100 cc. of digestion mixture.

RESULTS Caffeine when present in concentrations of 20 mgm. and 40 mgm. per 100 cc. of substrate showed absolutely no effect on the activity of any of the enzymes studied, namely, salivary and pancreatic amylases, pepsin, trypsin, and pancreatic lipase.

The coffee extract likewise was without effect on the proteolytic enzymes, pepsin and trypsin. It is recognized, however, that the concentration of hydrochloric acid necessary for the activity of pepsin doubtless altered the nature of the coffee extract, and that in our experiments, therefore, it was not the effect of the original constituents of the coffee that was determined but of the products formed by the acid treatment. But since this acidity is comparable to that of the gastric juice, in which medium pepsin acts *in vivo*, it may be assumed that coffee undergoes similar changes in the stomach and would produce a similar effect on the action of pepsin *in vivo*. The experiments showed, however,

a decided increase in the rate of digestion by both salivary and pancreatic amylase when coffee extract was present. This is shown in table 1. Since this favorable influence is not due to the caffeine present, it must be attributed to some other of the many constituents of coffee.

All determinations the results of which are compared were carried out in duplicate and at the same time under exactly the same conditions. Duplicate determinations showed an experimental error of less than 0.5 per cent so the variations recorded below, while not large, are certainly significant. The results given in the table are averages of several experiments. Table 1 also shows the results obtained with the pancreatic lipase. The activity of this enzyme was retarded when coffee extract was added to the digestion mixture, this effect being quite large when 40 cc. of the extract per 100 cc. of substrate were present. This retardation is doubtless due, at least in part, to the fact observed in these experiments, that the coffee infusion markedly decreased the stability of the olive oil emulsion used as substrate. This would obviously produce an unfavorable condition for the interaction of enzyme and fat molecules.

SUMMARY

1. Caffeine in concentrations of 20 mgm. and 40 mgm. per 100 cc. of substrate has no effect *in vitro* on the saccharogenic action of salivary and pancreatic amylases; nor does it affect the digestion of casein by pepsin and trypsin, or of olive oil by pancreatic lipase.

2. Coffee extract in the two concentrations studied does not affect the digestion *in vitro* of casein by pepsin or trypsin.

3. Coffee extract in the two concentrations studied increases the rate of digestion of starch by the salivary and pancreatic amylases.

4. Coffee extract in the two concentrations studied retards the digestion of olive oil by pancreatic lipase.

5. An improvement in the lipolytic method was made by using isopropyl alcohol instead of ethyl alcohol, and bromthymol blue as indicator instead of phenolphthalein in the procedure for the titration of the fatty acids formed.

REFERENCES

SABALITSCHKA, T. AND C. SCHULZE. Fermentforschung 8: 464, 1925.
OHNISHI, A. Sei-i-kwai Med. J. 47: 2, 1928.
HEIDE, E. AND E. SCHILF. Biochem. Ztschr. 213: 190, 1929.
ZLATAROV, A. AND I. D. POPOV. Ztschr. Untersuchung Lebensm. 73: 154, 1937.
SHERMAN, H. C. AND F. WALKER. J. Am. Chem. Soc. 43: 2461, 1921.

THE RÔLE OF THE BRAIN STEM IN ARTERIAL HYPERTENSION SUBSEQUENT TO INTRACRANIAL HYPERTENSION

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Cushing (1902) described the arterial hypertension which occurs immediately upon the experimental elevation of intracranial pressure to a level above that of the diastolic blood pressure. He further demonstrated that section of the vagus had no effect upon this phenomenon but that either section of the cervical spinal cord or the introduction of cocaine into the fourth ventricle obviated the blood pressure response. From these findings he concluded that the blood pressure regulating mechanism is a function of the medullary vasomotor centers and is released by the anemia induced by the elevation of the intracranial pressure to a level above that of the blood pressure.

Subsequently it was learned that pressor responses could be obtained by stimulation of certain portions of the central nervous system rostral to the pons. Ranson and Magoun (1939) in their monograph on the hypothalamus reviewed the experiments of others and added their own observations regarding the areas of the hypothalamus from which pressor responses could be elicited by stimulation. Hoff and Green (1936) and Crouch and Thompson (1936) described pressor responses on stimulation of discrete portions of the cerebral cortex. The possibility therefore arises that the pressor responses to increased intracranial pressure might originate, not in the medullary centers, but rather in these higher centers and thus the medullary centers might represent the final synapse, a position analogous to that of the anterior horn cell in the motor system.

While it is thus conceivable that the mechanism might originate as a suprapontine one, the possibility also arises that the origin of the pressor response might be in the medullary centers but the efferent arc extends rostrally through the brain stem. Chang and his collaborators (1937, 1940) and Sattler (1940) described a vago-supra-optic-posterior pituitary pressor mechanism upon stimulation of the central end of the sectioned vagus nerve.

The introduction of cocaine into the fourth ventricle could then obviate the pressor response to increased intracranial pressure in any one of three ways; the depression of the medullary centers, the interference with either ascending or descending pathways between these structures and pressor areas situated more rostrally, or by diffusion into the third ventricle and directly affecting the hypothalamic nuclei.

It therefore remains to be determined whether the medullary vasomotor centers are those primarily concerned in the blood pressure response to increased intracranial pressure and whether the connections—either ascending or descend-

ing—of these centers to higher pressor areas are necessary. To determine this studies were made of the blood pressure responses to increased intracranial pressure before and after decerebration and destruction of the medulla.

METHOD. Nine cats were studied. Surgical anesthesia was induced by intraperitoneal injection of 10 per cent sodium amytal (0.6 cc. per kgm.). A tracheal cannula was inserted, a midline scalp incision made and skin, galea, and muscles reflected. A trephination ($1\frac{1}{2}$ cm. in diameter) was performed over the right parieto-occipital area as far inferiorly and posteriorly as was possible while still avoiding the bony tentorium. Following radial incision and reflection of the dura, a fitted, threaded, metal cannula was inserted into the trephination and attached to a mercury manometer and writer and a pressure bottle containing physiological saline. Blood pressure recordings were obtained from the femoral artery by means of a mercury manometer and writer, the anti-coagulant being citrate. In all experiments, the animals were placed on artificial respiration with the onset of respiratory failure during the first intracranial pressure rise, and this artificial respiration was continued for the duration of the observations.

An initial record was taken of the blood pressure response to raised intracranial pressure. The cranial cannula was then removed and the animal was decerebrated. This was performed by introducing a blunt probe into the trephination below the occipital lobe, following the bony tentorium and then cutting the brain stem on the same plane as the tentorium. This resulted in a section either between the inferior and superior colliculi or through the superior colliculi. Cerebral edema occurred at this point but the trephination, by serving the purpose of a decompression, allowed this to recede. The cannula was then replaced and the pressure changes studied. The medulla was destroyed by maceration by means of a blunt probe introduced through the trephination.

The animals were sacrificed by intra-aortic injections of saline followed by 10 per cent formalin. In this manner the brain stem was fixed in situ and the damage wrought by maceration was more accurately determined.

RESULTS. Following decerebration the blood pressure response to an increased intracranial pressure was not only obtainable (fig. 1) but when compared with the results before decerebration was augmented both as to height and duration. Whereas prior to decerebration the blood pressure level exceeded the intracranial pressure level by a customary range of 15–40 mm. Hg the difference after decerebration was in the neighborhood of 30–90 mm. Hg.

The persistence of the blood pressure response after decerebration occurred in eight of the nine animals. In the first experiment attempted the cannula was replaced immediately after decerebration and without allowing the cerebral edema to subside. A spontaneous blood pressure rise occurred when the cannula was replaced. Subsequent intracranial injection of saline produced no further blood pressure response. In the remaining eight animals time was allowed for subsidence of the cerebral edema and in all instances adequate blood pressure responses were obtainable.

Maceration of the medulla to the level of the superior border of the inferior

olive resulted in the abolition of the blood pressure response (fig. 2). When the destruction included only areas rostral to this level the blood pressure response was obtainable. These results following maceration were obtained in all of the three animals upon whom this procedure was carried out. Whenever the blood pressure response had been obviated in the above manner it was still possible to produce an adequate blood pressure rise (to 200–220 mm. Hg) by the intravenous injection of adrenalin, thus indicating that peripheral mechanisms for the production of hypertension were still available.

DISCUSSION. The conclusions drawn from these results are that the vasomotor centers essential for the production of the vascular hypertension subsequent to intracranial hypertension extend no farther rostrally than the level of
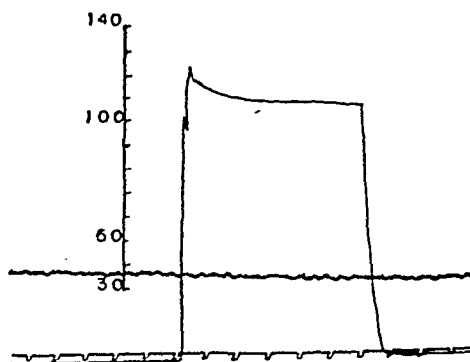
Fig. 1   Fig. 2

Fig. 1. Cat under amytal anesthesia and decerebrated. Upper tracing, blood pressure; lower tracing, intracranial pressure. Numbered scale in mm. Hg. Time interval 5 sec. Demonstrates, following decerebration, an adequate rise in blood pressure level upon elevation of the intracranial pressure level.

Fig. 2. Same cat under amytal, decerebrated and brain stem macerated to level of the superior border of the inferior olive. Scale values and time intervals as in figure 1. Demonstrates no rise in blood pressure level upon increasing the intracranial pressure level.

the superior border of the inferior olive and that the neuronal connections—whether afferent or efferent—of these centers with higher vasomotor centers are non-essential. Indeed the blood pressure rise is higher and the hypertension is longer maintained when these connections are severed.

These results are not inconsistent with the findings of Denny-Brown and Russell (1941) who noted that in concussing decerebrate preparations blood pressure rises occurred. Their experiments are not quite comparable to the ones here discussed because of the obviously more complicated situation in concussion, following which, as they showed, elevation of the intracranial pressure occurs only under certain conditions.

Dock and his collaborators (1942) studying renal hypertensive and controlled dogs found that crushing the brain stem rostral to the pons produced a

fall in blood pressure with a tendency to regain previous levels and still maintaining a distinct difference in levels between the hypertensive and controlled groups. Destruction of the cord, medulla and pons, however, yielded a sharp fall in blood pressure in both groups and without a significant difference in the blood pressure levels of hypertensive and control animals.

The decerebration studies reported here show that anatomical segregation of the medulla and pons from neural structures rostral to them does not obviate the increase in blood pressure due to intracranial hypertension. This indicates that neither anemia of the cortex and hypothalamus nor involvement of the vago-supra-optic mechanism can play an active part in this response since it persists after both the ascending and descending pathways have been severed.

Destruction of the medulla obviates the blood pressure response to increased intracranial pressure. Therefore, it may be concluded that the vasomotor centers essential for arterial hypertension subsequent to intracranial hypertension are situated in the medulla, and that the efferent pathways do not progress further rostrally in the brain stem than this level.

CONCLUSIONS

1. The blood pressure response to increased intracranial pressure depends upon the integrity of the medulla.

2. Neural structures rostral to the medulla are not necessary for this response.

3. Anatomical separation of the medulla and pons from higher structures enhances the response.

REFERENCES

(1) CHANG, H. C., K. F. CHIA, C. H. HSÜ AND R. K. S. LIM. Chinese J. Physiol. **12:** 309, 1937.

(2) CHANG, H. C., J. D. HUANG, Y. M. LÜ AND Y. C. TSANG. Chinese J. Physiol. **15:** 445, 1940.

(3) CROUCH, R. L. AND K. THOMPSON. Anat. Rec. **64:** (Suppl.) 11, 1936.

(4) CUSHING, H. C. Mitteil. a. d. Grenz Med. u. Chir. **4:** 773, 1902.

(5) DENNY-BROWN, D. AND W. R. RUSSELL. Brain **64:** 93, 1941.

(6) DOCK, W., F. SHIDLER AND B. MAY. Am. Heart J. **23:** 513, 1942.

(7) HOFF, E. C. AND H. D. GREEN. This Journal **117:** 411, 1936.

(8) RANSON, S. W. AND H. W. MAGOUN. Ergebn. Physiol. biol. Chem. u. exper. Pharmakol. **41:** 56, 1939.

(9) SATTLER, D. G. Proc. Soc. Exper. Biol. and Med. **44:** 82, 1940.

OBSERVATIONS ON THE CIRCULATION IN THE HIND LIMBS OF A DOG TEN YEARS FOLLOWING LEFT LUMBAR SYMPATHETIC GANGLIONECTOMY

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We have reported (1) simultaneous observations on the blood flow in the femoral arteries of denervated and innervated limbs. Control observations were made before and at various intervals of time following denervation by complete sympathetic ganglionectomy from the first or second lumbar ganglion to the end of the chain. It was found repeatedly that the blood flow on the denervated side was about twice that on the innervated side provided the operative procedures for applying the thermostromuhr units were done with the animal under local anesthesia. Under general anesthesia with ether the difference of blood flow was not apparent, the flow in the two sides becoming practically the same.

We were interested to determine how long this difference of blood flow would persist. In a series of experiments observations were made at different time intervals following lumbar sympathetic ganglionectomy. The longest interval was two years and ten months after sympathectomy and since the disparity of the flow of the two femoral arteries still persisted it seemed reasonable to conclude that the difference of blood flow in the hind limbs resulting from unilateral sympathetic ganglionectomy was permanent in the dog.

The present communication is concerned with observations which have indicated that the conclusion just mentioned is open to question. We shall present evidence obtained from the study of one animal indicating that, if a sufficient time after operation has elapsed, the increased flow in the femoral artery following sympathectomy may be reduced to approximately that in the control or innervated side.

METHODS AND RESULTS. As in other studies on blood flow the thermostromuhr was used. The operation for applying the units to the femoral arteries was done with infiltration anesthesia using procaine. The blood flow in both femoral arteries was observed simultaneously. Lumbar sympathetic ganglionectomy was done on the left side using sterile technic and with the animal under general anesthesia.

We desired to make observations on some of the animals of our original series after several years had elapsed. However, since we became interested in other investigations, further observations on the sympathectomized dogs were postponed and more than nine years had passed before we returned to the problem. By that time only one of the original group of dogs remained. Examination of data obtained on this animal indicated that in a control observation made before

sympathectomy the flows in the right and left femoral arteries were respectively 108 and 77 cc. each minute.   Fifteen days after sympathectomy the blood flow on the right and left sides was respectively 99 and 297 cc. each minute.   Then again nearly eleven months after the operation the flow on the right side was 188 cc. and that on the left or denervated side was 377 cc. each minute.   When the blood flow in the femoral arteries of this remaining dog was measured more than nine years after sympathectomy the flow in the right femoral artery was 112 cc. and that in the left was 125 cc. each minute (table 1).

In seeking an explanation of this unexpected result it was decided to observe the effects of intravenous injections of epinephrine and histamine.   It was found that the flow in the femoral artery of the innervated limb was practically unaffected by the doses of epinephrine used but the sympathectomized side showed a maximal response since the flow appeared to be completely shut off.   Following injections of effective doses of histamine the results on the two sides were again

TABLE 1

Blood flow in femoral arteries of trained dog before and after left lumbar sympathetic ganglionectomy

OBSERVATION	FLOW	
	Right femoral	Left femoral
	cc. per min.	*cc. per min.*
Before sympathectomy I. A. P.*.............................	108	77
15 days after sympathectomy I. A. P.........................	99	297
10 months, 25 days after sympathectomy I. A. P.............	188	377
9 years, 2 months, 1 day after sympathectomy I. A. P........	112	125
Same except under pentobarbital sodium anesthesia..........	131	140
10 years, 2 months, 22 days after sympathectomy I. A. P......	91	110

* I. A. P. = Infiltration anesthesia with procaine.

very different and quite the reverse of what followed injections of epinephrine. The flow on the denervated side was only slightly affected whereas that on the innervated side was markedly increased (table 2).

A plethysmograph was later placed on each hind foot, after which the same drugs were given.   The changes of the volume of the foot reflected what had occurred when the blood flow was recorded.   There was a transient decrease of the volume of the innervated foot with small doses of epinephrine but a very prolonged decrease of the volume of the denervated foot.   When histamine was given the denervated foot showed only a slight increase while the innervated foot increased markedly in volume.   The difference of the response of the two limbs to vasoconstrictor and vasodilator drugs raised the question of the possibility that a change had occurred in the walls of the blood vessels of the denervated side.   We conceived of the vessels as being inclosed by thick connective tissue which might prevent dilatation but yet permit contraction.   At the suggestion of Doctor Barker a toe was removed from each hind foot of this animal and the tissues were studied microscopically.   Sections of the tissues of the two toes

were placed alternately on slides in a certain sequence. The observer was unaware of the identity of the sections. He was asked to determine whether there was any difference that he could detect between the vessels of the successive sections. Several of our colleagues reported that the arterioles seen in certain

TABLE 2

Effect of epinephrine and histamine on blood flow in right and left femoral arteries nine years, two months and one day after left lumbar sympathetic ganglionectomy

DRUG	RIGHT ARTERY FLOW	LEFT ARTERY FLOW	ANESTHETIC
Epinephrine, 0.05 cc. 1:1,000 in 15 seconds	Slight increase, slight decrease	Zero flow for 1 minute	Local infiltration anesthesia with procaine hydrochloride
	Slight increase, slight decrease, return to control	Zero flow for 1 minute	
Histamine, 0.01 mgm. per kgm.	240 per cent increase	90 per cent increase	
	74 per cent increase	3 per cent increase	Pentobarbital sodium anesthesia
Epinephrine, 0.05 cc. 1:1,000 in 15 seconds	Slight increase, slight decrease	Zero flow for 2 minutes	
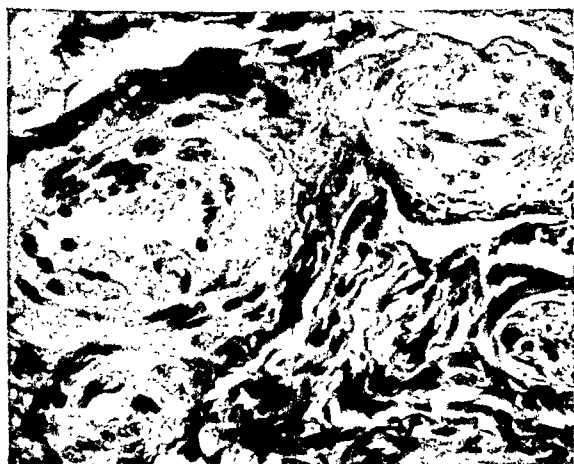
Fig. 1   Fig. 2

Fig. 1. Tissue from a toe taken from the left foot of a dog more than nine years after sympathetic ganglionectomy on the left side.

Fig. 2. Same as figure 1 except the tissue was from the right or control side.

cf the sections were markedly thickened as compared with what was seen in the other sections. It was found that hypertrophy of the arterioles was present on the denervated side and was confined to the media or muscularis coat of the vessels (fig. 1). The vessels of the control side (fig. 2) did not show hypertrophy.

A year later, or ten years, two months and twenty-two days after sympathetic ganglionectomy, the flow in the two hind limbs was again observed. At that

time the flow in the femoral artery of the innervated limb was 91 cc. and that in the denervated limb was 110 cc. each minute. The effects of injections of epinephrine and histamine were the same as already described. Under pentobarbital sodium anesthesia the effect of epinephrine and histamine on the blood flow in the two limbs was observed with results similar to those described before in this report.

In addition a small artery less than a millimeter in diameter was exposed in both legs just below the knee. The behavior of these arteries was observed before and after administration of epinephrine. The response, a marked constriction of the artery on the denervated side, was much greater than that of the control side. Both arteries were then cut so that a vigorous stream of blood issued forth. After epinephrine was given, the vessel on the denervated side constricted sufficiently to shut off the blood flow whereas the vessel on the control or innervated side continued to bleed profusely. That the lack of flow on the denervated side was not the result of formation of a clot was demonstrated by the resumption of profuse bleeding as soon as the effect of the injected epinephrine was dissipated.

Thus it appears that both the arterioles and the small arteries of the denervated side were more sensitive to epinephrine than those of the innervated side. Observations were made on the animal under local and under general anesthesia with pentobarbital sodium until death. All observations were confirmatory of those made a year previously as were the findings on additional sections made from tissues removed from the two legs. A difference of the blood vessels of the two sides was not apparent above the knee of the animal. At necropsy a careful search of the left lumbar tissues was made but there was no gross evidence of sympathetic ganglia or sympathetic nerves being present.

COMMENT. At the outset it must be emphasized that all the observations recorded in this report were made on a single animal but at the same time it should be pointed out that a better controlled experiment is difficult to devise. One hind leg of a given animal should offer the ideal control on the other hind leg. The number of variables introduced should be minimal. Furthermore it is not frequently that we are permitted observations on an experimental animal over so long a period of years. Therefore we may be excused for having discussed the findings on a single animal at considerable length.

The question at once arises as to the cause of the hypertrophy of the walls of the arterioles. Two possible explanations have been considered. 1. The hypersensitivity of the vessels of the denervated limb was sufficiently great to suggest the possibility of a vigorous response of these vessels to the animal's own secreted epinephrine, which over a period of years might result in a work hypertrophy of the muscles of the vessels. 2. It is common knowledge that sympathectomy impairs the tone of blood vessels. Consequently the vascular musculature is subjected to stretching which, if resisted over long periods of time, might result in muscular hypertrophy in the walls of the involved vessels.

Quite obviously the answer as to what brought about the difference in the small vessels of the two limbs must await the results of more experiments.

SUMMARY AND CONCLUSIONS

Observations have been made on the blood flow in the femoral arteries of a dog at intervals over a period of ten years and two months.   Blood flow was measured in both femoral arteries simultaneously under infiltration anesthesia before sympathetic ganglionectomy and fifteen days; ten months, twenty-five days; nine years, two months and ten years, two months after sympathectomy. Whereas the flow was twice as great in the left or sympathectomized limb as it was in the right or control limb fifteen days and also ten months and twenty-five days after sympathectomy, the flow was almost the same in the two limbs nine and also ten years after sympathectomy.

That the vessels of the sympathectomized leg were profoundly hypersensitive to epinephrine more than nine and ten years following sympathectomy was shown by a small injection of epinephrine which caused such a marked constriction of the vessels of the sympathectomized side that the flow was zero for at least two minutes.   The flow on the control side taken at the same time was only slightly altered.

Simultaneous plethysmographic records of the two hind feet further confirmed the observations on blood flow since there was a transient decrease of the volume of the innervated foot but a very prolonged decrease of that of the denervated foot in response to small doses of epinephrine.

Histologic examination of a toe from each hind foot showed that the arterioles of the left or sympathectomized foot had undergone hypertrophy which was confined to the muscularis coat of the vessels.   The vessels of the control side did not show hypertrophy.

REFERENCE

(1) HERRICK, J. F., H. E. ESSEX AND E. J. BALDES.   This Journal **101**: 213, 1932.

THE EFFECT OF SODIUM BICARBONATE ON GASTRIC SECRETION[1]

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The effect on gastric secretion of $NaHCO_3$ administered by mouth has been controversial since Claude Bernard (1) first observed that it stimulated secretion. The majority of comparatively recent clinical reports give the impression that it induces an increase in acid secretion following the initial period of neutralization. Thus, Spencer et al. (2) found by fractional gastric analysis that 0.65 to 5.0 per cent solutions of $NaHCO_3$ produced higher curves of acidity than were obtained in controls. Crohn (3) reported that 2 grams of $NaHCO_3$ given after a test meal increased the acidity shown by fractional analysis. Lockwood and Chamberlin (4) noted that the inclusion of 4 grams of $NaHCO_3$ in the test meal raised the acidity in 50 per cent of their cases. These reports are largely the basis for the concept of a "rebound" or "secondary" secretion of acid which is frequently mentioned in current literature.

The nature of this "rebound" is not clear. Fractional analysis measures *concentration* of acid. Without minimizing the importance of acid concentration, we raise the question of whether the "rebound" acid secretion is an active stimulation or simply a passive rise in acid concentration. For example, it is known that $NaHCO_3$ decreases the emptying time of the stomach (5). In view of this and the fact that parietal cell secretion is constantly high in acid concentration, the increase noted by fractional analysis may be the result of diminished dilution of secretion which enters the stomach when it is more nearly empty.

The answer to the above question thus depends primarily on a comparison of the *amounts* of acid secreted during an entire digestive period *a*, under control conditions, and *b*, under conditions in which the "rebound" is elicited. Animal experimentation is necessary to obtain such data. The nearest approach to this type of data in the literature is Boyd's report (6). Two Pavlov pouch dogs were fed $NaHCO_3$ daily for more than 2 months. The mean rate of secretion and the mean acidity of the pouch collections were increased during the $2\frac{1}{2}$ hours immediately following the test meal. However, several factors complicate interpretation of these data: *a*, the daily period of secretion observed was relatively short; *b*, an appraisal of the effect of the intentional variation (inclusion of table scraps, etc.) in the daily meal, which served as the test meal, is difficult; *c*, the

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average data reported include days on which gastro-intestinal upsets (diarrhea, vomiting) occurred. Therefore, in the absence of sufficient data for an adequate evaluation, we have sought to determine the effect of $NaHCO_3$ on gastric secretion by use of the Cope pouch dog.

EXPERIMENTAL. This is apparently the first report of the experimental use of the Cope gastric pouch dog (7) and, therefore, the procedures developed in this laboratory and used in these experiments will be described in detail. A Cope pouch is made from most of the anterior wall of the body of the stomach by a two-stage operation which leaves the vagal nerve supply intact. Unlike most pouches, the stoma lies high on the dog's flank and no collection vessels, bandages, or restraints are required, since the pouch hangs dependent within the abdomen and serves as its own collection vessel. However, these conditions require an antacid in the pouch to prevent peptic ulceration. Measurement of the amounts of chloride, base, acid, and of the volume secreted during any interval desired may be made by using the technic described below, which permits continuous 24 hour study day after day.

The *control routine* employed was as follows: The pouch was emptied with a catheter and syringe at 8 and 11 a.m. and at 2 and 5 p.m. The test meal was fed immediately after the 8 a.m. collection. The three daytime collections following the meal, and the collection at 8 a.m. the following morning (night secretion) constituted a 24 hour collection. After each emptying a measured amount of aluminum hydroxide gel of known composition was placed in the pouch. Water was allowed ad libitum day and night. At the end of each week an arterial blood sample was taken before feeding the meal which included, on that day, one tablespoonful of brewer's yeast. Red and white cell counts, cell volume (8), O_2 and CO_2 capacity of whole blood (9), and plasma chloride (10) were determined.

The *test meal* given at 8 a.m. served not only as the stimulus of secretory activity, but also as a vehicle for the return of electrolytes lost via the pouch, and as an adequate diet. A weighed amount of a commercial dog food[3], sufficient to maintain weight and normal activity, was mixed daily at 4 p.m. with 50 cc. of 1 N HCl and an adequate amount of NaCl. The amount of the latter was determined by following the blood chloride and CO_2 capacity, after which it was kept constant from day to day. The meal buffered the HCl and was more palatable than when all necessary chloride was returned as NaCl. Each meal was consumed within 10 minutes and all animals had voracious appetites.

The *pouch antacid* was aluminum hydroxide gel[4] diluted 1:1 by volume with water. Individual animals required 25 to 50 cc. of this dilution to neutralize the acid secreted during the collection period. Aluminum hydroxide gel was used because: *a*, it is more efficient than $NaHCO_3$ in preventing ulceration of the Cope pouch; *b*, it maintains a pH within the pouch nearer the reaction of normal gastric contents; *c*, it is not absorbed; and *d*, it does not interfere with the electrolytic determination of base.

Each pouch collection was filtered through a coarse fritted glass filter and the filtrate analyzed in duplicate for chloride (10) and base (11). Similar determinations were made on each lot of diluted aluminum hydroxide gel. Control studies showed that no appreciable error was introduced by filtration or by the presence of aluminum in the filtrate. Quantitative determinations (12) showed only *negligible traces of aluminum in the cathode vessel in the electrolytic* determination of base.

The volume of the original pouch collection in liters multiplied by the concentrations of chloride and base in milliequivalents per liter gave the amounts in milliequivalents of these constituents in the collection. From these values were subtracted the amounts of chloride and base initially present in the antacid to give the total chloride and total base of the *pouch secretion*. The amount of free acid secretion was estimated by subtracting the secretion total base from the
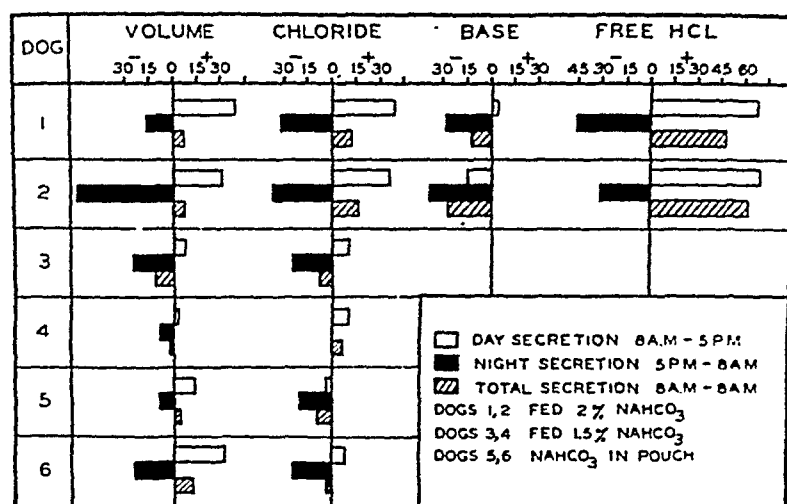
Fig. 1. The average per cent of change from controls in secretion of the Cope pouch when NaHCO₃ is fed by stomach tube, or when 4 per cent NaHCO₃ is placed in the pouch.

secretion total chloride. The *secretion volume* was obtained by subtracting the volume of antacid placed in the pouch from the volume of *pouch collection*.

Five Cope pouch dogs accustomed to the stomach tube were used. The first series of experiments consisted of volume and total chloride determinations on the pouch collection from dogs 3 and 4 on control routine for 30 days, with the exception that from the 7th to 18th day each dog received by stomach tube 50 cc. of 1.5 per cent NaHCO₃ solution daily at 9 and 11 a.m. and 2 p.m.

In a second series, dogs 1 and 2 each received 50 cc. of 2 per cent NaHCO₃ solution daily at 9 and 11 a.m. and 2 p.m. from Monday morning through Friday afternoon. A week of control and a week of NaHCO₃ feeding were alternated twice, giving 4 consecutive weeks of study. As a further control, the effect of administering 50 cc. of tap water, instead of the NaHCO₃ solution, was determined by a fifth week of observation. Complete analyses were made on the collections of this series.

The effect of NaHCO₃ in direct contact with the *pouch* mucosa was observed,

TABLE 1

Effect of feeding sodium bicarbonate on gastric secretion in the Cope pouch*

TEST WEEK	COLLECTION PERIODS	SECRETION VOLUME	TOTAL CHLORIDE AMT. SECRETED	TOTAL BASE AMT. SECRETED	FREE HCl AMT. SECRETED
Dog 1 (8 mos. post-operative)					
		cc.	*m.eq.*	*m.eq.*	*m.eq.*
2/1–2/7/42 control	Day	152	29.6	13.0	16.6
	Night	91	18.0	14.4	3.6
	24 hours	243	47.6	27.4	20.2
2/8–2/14/42, fed NaHCO₃	Day	211	42.8	12.7	30.1
	Nights	30	10.3	8 6	1.7
	24 hours	241	53.1	21.3	31.8
2/15–2/21/42, control	Day	159	31.0	14.4	16.6
	Night	83	17.8	14.1	3.7
	24 hours	242	48.8	28.5	20.3
2/22–2/28/42, fed NaHCO₃	Day	220	40.6	16.3	24.3
	Nights	.57	14.1	12.2	1.9
	24 hours	277	54.7	28.5	26.2
3/23–3/28/42, fed H₂O	Day	187	33.8	15.0	18.8
	Night	114	22.1	18.5	3.6
	24 hours	301	55.9	33.5	22.4
Dog 2 (7 mos. post-operative)					
2/1–2/7/42, control	Day	90	18.8	7.6	11.2
	Night	54	11.2	9.5	1.7
	24 hours	144	30.0	17.1	12.9
2/8–2/14/42, fed NaHCO₃	Day	112	25.0	6.4	18.6
	Night	14	5.4	4.6	0.8
	24 hours	126	30.4	11.0	19.4
2/15–2/21/42, control	Day	103	20.5	7.5	13.0
	Night	11	4.6	4.0	0.6
	24 hours	114	25.1	11.5	13.6
2/22–2/28/42, fed NaHCO₃	Day	139	28.4	6.7	21.7
	Night	11	4.6	4.4	0.2
	24 hours	150	33.0	11.1	21.9
3/23–3/28/42, fed H₂O	Day	114	21.3	6.9	14.4
	Night	32	7.0	6.2	0.8
	24 hours	146	28.3	13.1	15.2

* Each figure is the average of three values obtained on three consecutive days during the week indicated.

in a third series, by volume and total chloride determinations on the collections from dogs 5 and 6 (dog 5 was no. 3 of the first series). After a week of control

with aluminum hydroxide gel in the pouch, 3 weeks of study were carried out in which 4 per cent $NaHCO_3$ solution was substituted for the gel. This was followed by 3 weeks of control with the gel again in the pouch.

RESULTS. No gastro-intestinal disturbances (vomiting, diarrhea) occurred in these studies. The data of individual days are too extensive to be shown, but they agree closely with the averages reported.

In the first series, during the feeding of 1.5 per cent $NaHCO_3$, small increases in volume (247 to 260 and 114 to 117 cc.) and in total chloride (47.8 to 52.1 and 25.7 to 28.0 m.eq.) were seen in both dogs during the first 9 hours after the test meal. The remaining 15 hours showed a decrease of volume in both dogs (285 to 214 and 56 to 51 cc.) with a decrease in total chloride (51.3 to 39.1 m.eq.) in one, while the other was unchanged (14.7 m.eq.). The average net result for 24 hours was a decrease in the volumes of secretion in both dogs (532 to 474 and 171 to 168 cc.), a decrease of total chloride (99 to 91 m.eq.) in one, and an increase (40 to 43 m.eq.) in the other. These variations are shown as percentages of change in figure 1, dogs 3 and 4.

Averages of the data of the second series (dogs 1 and 2, receiving 2 per cent $NaHCO_3$) are shown in table 1. These values are averages of 3 consecutive days, usually Wednesday, Thursday and Friday, and represent the results of that week. "Day" collection periods are the sum of the 3 separate collections made at 11 a.m. and at 2 and 5 p.m. The feeding of $NaHCO_3$ produced increases in volume, total chloride and free acid during the first 9 hours and were accompanied, with one exception, by slight decreases in total base. Despite a general decrease of all values in the following 15 hours, there was a net 24 hour increase in volume, total chloride and free acid, and a decrease in total base. The percentile differences in the values of the averaged 2 weeks of $NaHCO_3$ feeding from the averaged 2 weeks of control are shown in figure 1, dogs 1 and 2. The feeding of water instead of a solution of $NaHCO_3$ produced values only slightly higher, in a few instances, than controls. The distribution of the averaged secretory values of this series into individual collection periods is shown in figure 2. Increases in the values of the earlier periods (1 and 2) and decreases in the night period (4) are clearly evident. Table 2 shows no significant change in the hematology of these animals. The usual reciprocal relationship between plasma chloride and carbon dioxide capacity during $NaHCO_3$ feeding is seen in the small decrease (average 5.8 and 1.8 m.eq.) in the former and small increase (average 4.2 and 4.4 vol. per cent) in the latter.

The third series, in which 4 per cent $NaHCO_3$ was substituted for aluminum hydroxide gel in the pouch, showed changes similar to those seen when $NaHCO_3$ was fed. The average changes are shown as dogs 5 and 6 in figure 1. The pH of pouch contents at the time of collection varied between 5.3 and 7.8 (Beckman glass electrode) when $NaHCO_3$ was in the pouch and between 3.9 and 4.3 when aluminum hydroxide gel was present.

DISCUSSION. The objective of the technic used in these studies was to approach normal physiological conditions as closely as possible while allowing *continuous* observation of the secretory activity, rather than observation for a

few hours, following a test meal. In this direction are the following: *a*. The Cope pouch dog lives more normally than a pouch dog which requires bandages,
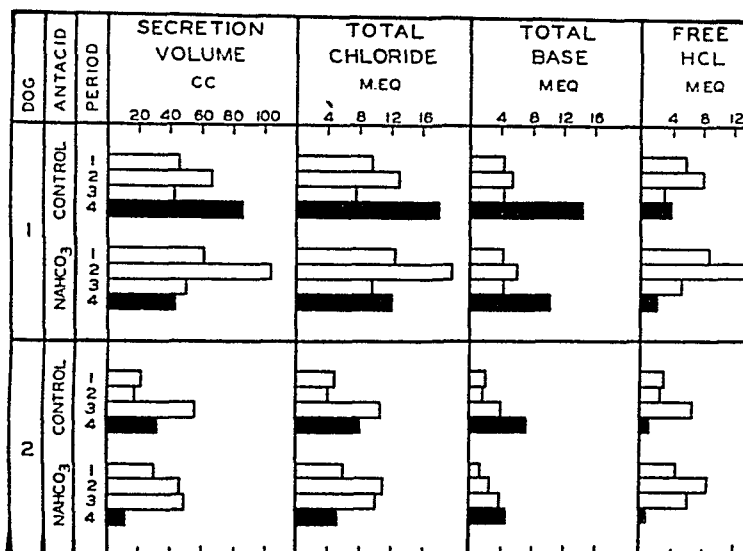
Fig. 2. The average secretory response of the Cope pouch during two weeks of feeding 50 cc. of 2 per cent NaHCO₃, t.i.d., compared with the average response during two weeks of control. The comparison is by collection periods 1 to 4, which are respectively, from 8–11, 11–2, 2–5 o'clock in daytime (open bars) and at night from 5 p.m. to 8 a.m. (black bars).

TABLE 2

Hematology and blood chemistry of the Cope pouch dog during the experiments shown in table 1

DATE	R.B.C.	W.B.C.	CELL VOL.	O₂ CAP.	Hb.	CO₂ CAP.	PLASMA CHLORIDE	DOG WT.
	×10⁶	×10³	*per cent*	*vol. per cent*	*gms.*	*vol. per cent*	*m.eq./l.*	*kgm.*
				Dog 1				
2/ 7/42	5.74	7.75	46.5	23.0	17.1	41.2	111.6	10.6
2/14/42	6.75	14.10	48.0	22.6	16.9	45.2	106.8	10.7
2/21/42	7.47	7.50	48.0	23.1	17.2	40.2	115.8	10.5
2/28/42	6.75	10.85	48.0	23.0	17.2	44.5	109.0	11.3
3/28/42	6.45	10.55	47.5	23.2	17.3	48.0	114.0	11.0
				Dog 2				
2/ 7/42	6.23	20.45	43.0	21.6	16.2	45.6	113.0	16.0
2/14/42	7.12	12.75	44.0	20.2	15.1	49.0	111.4	15.8
2/21/42	6.92	8.40	44.0	21.3	15.9	49.7	114.8	15.4
2/28/42	6.68	11.30	44.0	21.6	16.2	55.2	112.8	15.4
3/28/42	6.19	13.00	43.0	21.5	16.1	47.5	112.4	14.6

collection vessels, and restraints. *b*. A bulk of material is present in the pouch, as in the normal stomach during digestion, and its pH is not far removed from that of normal gastric contents. This avoids the abnormal condition of a relatively empty pouch in which the mucosa is in constant contact with a highly

corrosive, acid secretion. *c.* The test meal is not an interposed factor in the animal's normal routine, but is a part of that routine. *d.* Saline injections, known to influence secretion (13), and the inclusion of NaCl in the drinking water, to maintain acid-base balance, are avoided. *e.* The use of aluminum hydroxide gel within the pouch eliminates disturbances of the acid-base balance which may attend use of absorbable antacids (14).

The values obtained by this method of study may deviate slightly from absolute values: *a.* Aluminum hydroxide, a protein precipitant, removes most of the protein from the filtrate analyzed. Komarov and Komarov (15) have shown that pepsin is precipitated. *b.* The possibility of partial absorption of pouch contents is present even though the stomach is known to absorb most substances poorly. This possibility is common to all pouch studies. However, chloride appears to be unabsorbed by the gastric mucosa (16) and our studies support this observation. Significantly, we find that the amounts of the constituents secreted during a 9 hour control period do not vary when collections from the pouch are made hourly or at 3 hour intervals. Further, the values during control periods are reproducible from week to week with only slight variations. Those factors which may tend to introduce minor errors largely cancel out when the effects of a variable are determined by comparison of the differences between control and experimental values.

The effects of NaHCO₃ on the pouch secretion may be evaluated by considering figure 1. In all 6 cases the volumes during the first 9 hours showed increases ranging from 2.5 to 38 per cent. At the same time 5 dogs showed increases in total chloride which paralleled the volume changes, while dog 5 showed a slight decrease. To offset these general increases there were in every case, except the unchanged total chloride of dog 4, decreases at night which ranged in volume from 8.8 to 61 per cent, and in chloride from 20 to 37 per cent. The net results for 24 hours ranged from insignificant changes to marked increases in dogs 1 and 2. The daytime increases were characterized by the appearance of relatively larger amounts during the earlier periods of collection as seen in figure 2. This shift was typical of every case in the three series.

Further changes effected by NaHCO₃ are seen in the total base and free HCl variations of the second series. Figure 1, dogs 1 and 2, shows that there are significant net decreases of 12.5 and 38.0 per cent in total base, with marked net increases of 47 and 62.5 per cent in free acid. Thus in these experiments NaHCO₃ acts to elicit a higher percentage of the total chloride as free acid. This meets the need of more free acid to neutralize the NaHCO₃.

It becomes evident now that neither fractional gastric analyses nor pouch studies for a few hours following a test meal provide adequate data to reveal the effect of antacids on gastric secretion. Previous reports have shown that an increase in *acid concentration* may occur. Our data show at least two factors contributing to the "rebound" effect produced by NaHCO₃: *a.* The *amount* of acid secreted is increased. *b.* A shift occurs in secretory activity to greater amounts at earlier periods after the test meal. Both factors may operate in a given case, but our data show further, that even when *a* is relatively small, *b* may still be quite marked.

Considered as a whole these data suggest that the "rebound" acid concentration observed by fractional analysis after the ingestion of $NaHCO_3$ results primarily from the shift in gastric secretory activity. This brings a greater part of the secretion elicited by a test meal into the stomach at an earlier period and is accompanied by an increase in the amount of acid secreted. Since the stomach empties more rapidly under the influence of $NaHCO_3$, the factor of diminished dilution, as originally suggested, may then further increase the acid concentration.

The mechanism of the changes in gastric secretion observed in this study awaits clarification. Among the possibilities are the following: Sodium bicarbonate may cause secretory stimulation directly by local action on the mucosa, or indirectly by removal of acid inhibition (13) through neutralization. Or, perhaps, the small changes in total chloride and CO_2 capacity of the blood may be significant. Finally, the possibility of a humoral mechanism cannot be excluded. The similarity of the effects seen when the $NaHCO_3$ is fed and when it is placed in the pouch indicates that the mechanism involves more than a strictly local action on the parietal cells.

SUMMARY

Changes produced in gastric secretion by a 1.5 and a 2 per cent solution of $NaHCO_3$ administered three times daily in doses of 50 cc. by stomach tube, or a 4 per cent solution placed directly in the pouch were studied by use of a new technic employing the recently described Cope pouch dog.

Sodium bicarbonate produced an increase in the gastric secretory activity during hours immediately following a test meal, with a partial compensatory decrease during later hours. The role of these factors in "rebound" or "secondary" gastric acid secretion, as observed in fractional gastric analysis, is discussed.

REFERENCES

(1) BERNARD, C. Leçons de Physiologie Operatoire. Paris, 1879, 570.
(2) SPENCER, W. H., G. P. MEYER, M. E. REHFUSS AND P. B. HAWK. This Journal 39: 459, 1915.
(3) CROHN, B. B. Am. J. Med. Sci. 155: 801, 1918.
(4) LOCKWOOD, B. C. AND H. G. CHAMBERLIN. Arch. Int. Med. 32: 74, 1923.
(5) VAN LIERE, E. J. AND G. K. SLEETH. Am. J. Digest. Dis. 7: 118, 1940.
(6) BOYD, T. E. This Journal 71: 455, 1924.
(7) COPE, O., C. E. MACMAHON, A. HAGSTROMER AND R. H. THOMPSON. Arch. Surgery 40: 717, 1940.
(8) WINTROBE, M. M. Am. J. Med. Sci. 185: 58, 1933.
(9) VAN SLYKE, D. D. AND J. M. NEILL. J. Biol. Chem. 61: 523, 1924.
(10) VAN SLYKE, D. D. AND J. SENDROY. J. Biol. Chem. 58: 523, 1923.
(11) KEYS, A. J. Biol. Chem. 114: 449, 1936.
(12) EVELETH, D. F. AND V. C. MYERS. J. Biol. Chem. 113: 449, 1936.
(13) IVY, A. C. Surgery 10: 861, 1941.
(14) ADAMS, W. L. Arch. Int. Med. 63: 1030, 1939.
(15) KOMAROV, S. A. AND O. KOMAROV. Am. J. Digest. Dis. 7: 166, 1940.
(16) SHAY, H. J., J. GERSHON-COHEN AND S. FELS. Am. J. Digest. Dis. 6: 361, 1939.

STIMULATION OF GASTRIC SECRETION BY NEURINE[1,2]

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Intramuscular injection of an extract of yeast stimulates gastric secretion in dogs according to Williams, Cox and Nash (1). Additional (unpublished) work has confirmed the original suggestion that the active agent is not histamine. Attempts to isolate this yeast principle have been unsuccessful.

Another approach to the problem of identifying the active yeast principle was by testing compounds known or suspected to be present in yeast. Of substances

TABLE 1

Effect of injection of 2.5 mgm. of neurine bromide (= 1.5 mgm. of neurine base) per kilo body weight on gastric secretion in dogs

PERIOD	VOLUME	FREE HCl	TOTAL ACID
	cc.	*meq.*	*meq.*
30 minutes preceding injection........	8.2 ± 1.4	0.10 ± 0.04	0.24 ± 0.08
First 30 minutes after injection.......	42.2 ± 4.2	1.30 ± 0.19	2.00 ± 0.29
Second 30 minutes after injection.....	31.3 ± 3.5	0.93 ± 0.14	1.47 ± 0.23
Third 30 minutes after injection......	19.0 ± 2.3	0.40 ± 0.08	0.57 ± 0.13

so far tested, including choline chloride, beta-alanine, glutathione, and neurine bromide (Hoffman-La Roche), only the last was definitely active. All substances tested were injected intramuscularly in a dose of 2.5 mgm. per kilo body weight.

Data of 17 experiments with neurine bromide on 4 different dogs with gastric fistulas are summarized in table 1, which also records the probable errors of the means.

We have shown previously (1) that there is no significant increase in volume, free hydrochloric acid, or total acid following control injections of 0.9 per cent sodium chloride in such dogs. However, after injecting neurine increases do occur in all three values. When the increase is compared with the error of the mean, using the table of t given by Fisher and Yates (2), the probability of the change being due to chance is less than 0.01 in every comparison during the first and second 30 minute periods following the injection.

The increase of total acid in the first 30 minute period after injection of neurine is comparable with that obtained following a dose of about 0.01 mgm. of

histamine per kilo body weight, which has been found previously to be just above the threshold dose. In 6 experiments we have injected a smaller dose of neurine bromide (1.5 mgm. per kilo body weight) with results which are not statistically significant. The barely effective dose of neurine (which may be taken as 1.5 mgm. of neurine hydroxide per kilo body weight) is, therefore, about 150 times as large as that of histamine base.

It should be noted, however, that the effect of neurine is much more prolonged so that at the threshold dosage stated the total increase in volume and acid are more than double the increments after histamine. The prolonged action of neurine cannot be due to reabsorption from the gastric juice, since the latter was removed regularly by catheter.

The only report we have found of collection of gastric juice following injection of neurine is that of Miyazaki (3), who injected subcutaneously 25 mgm. or more of neurine per kilo body weight in order to demonstrate the subsequent presence of neurine in the gastric juice. He gave no data on the volume or acidity of secretion, however.

SUMMARY

Neurine, as bromide, when injected intramuscularly in dogs stimulates the secretion of fluid and acid by the stomach. The threshold dose of neurine base is about 150 times larger than that of histamine, but its effect is more prolonged.

In approximately the same dose of 2.5 mgm. per kilo body weight, choline chloride, beta-alanine and glutathione were ineffective.

REFERENCES

(1) WILLIAMS, E. F., JR., W. W. COX AND T. P. NASH, JR. This Journal 131: 378, 1940.
(2) FISHER, R. A. AND F. YATES. Statistical tables. p. 26, Oliver and Boyd, London, 1938.
(3) MIYAZAKI, M. J. Biochem. 19: 329, 1934.

ANAEROBIC SURVIVAL OF ADULT ANIMALS[1,2]

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The brain is regarded chiefly as an aerobic organ because anoxia causes rapid loss of cerebral functions.   Cerebral energy may, however, be obtained anaerobically as the brain is able to split carbohydrate to lactic acid.   Previous work on the brain of newborns of several mammalian species (1, 2) reveals that energy obtained by glycolysis is effective in supporting cerebral functions for a considerable period of anoxia.   Injections of iodoacetate and fluoride (3) were found to shorten the survival period in nitrogen by preventing the breakdown of carbohydrate to lactic acid.   It was concluded that though the rate of glycolysis in the newborn is slower than in the adult this slow rate appears adequate to support the lower metabolic demands of the infant brain for a considerable period of time in the absence of oxygen.   It is the purpose of the present investigation to study this anaerobic production of cerebral energy in adult dogs and cats and to evaluate its significance for survival in these animals with the relatively high rate of cerebral metabolism characteristic of adults.

METHOD.   *Eight per cent oxygen in nitrogen was used in most of the experiments on dogs because the degree of anoxia produced by this gas mixture results in a survival period of at least one hour in these animals when anesthetized with pentobarbital, 25 mgm. per kilo.*   After trying various doses of iodoacetate from 25 mgm. to 49 mgm. per kilo, a dose of 32 mgm. was found to be most suitable because it permitted survival of sufficient duration when the anesthetized dogs were respiring air.   In all instances the persistence of respiratory movements was employed as the criterion of survival.   Three groups of dogs were used: the first group was subjected to the respiration of 8 per cent oxygen, the second to the injection of iodoacetate and the third to anoxia after the injection of iodoacetate.

Cats anesthetized with pentobarbital, 25 mgm. per kilo, injected intraperitoneally were used to determine the effect of 11 per cent and 8 per cent oxygen on brain lactic acid.   Three groups of animals were studied: the first were controls, the other two respired either 11 per cent or 8 per cent oxygen for one hour. The brain was exposed through scalp and cranium.   Then each animal received a slow intravenous injection of iodoacetate, 75 mgm. per kilo, in an attempt to inhibit glycolysis.   Samples of brain and blood were taken and their lactic acid contents were determined by the method of Friedemann, Cotonio and Shaffer (4).

In another series of observations on dogs the effects either of the respiration of 8 per cent oxygen or the injection of 32 mgm. per kilo of iodoacetate on cerebral

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arterio-venous oxygen differences were studied. The blood was analyzed for oxygen by the method of Van Slyke and Neill (5).

RESULTS. Table 1 summarizes the effects of the respiration of 8 per cent oxygen in nitrogen and of iodoacetate injection on the survival period. It is evident that the combined effects of anoxia and iodoacetate greatly shorten the survival period.

Table 2 discloses that with the respiration of 11 per cent oxygen the concentration of cerebral lactic acid rises above the control value. With 8 per cent

TABLE 1

Survival time of adult dogs with 8 per cent oxygen, iodoacetate and both

CONDITION	NUMBER OF DOGS	SURVIVAL TIME	
		Range	Average
		minutes	*minutes*
8% oxygen..	5	60–271	151
Iodoacetate (25–49 mgm./kgm.)...................	17	49–240	127
8% oxygen + Iodoacetate (25–49 mgm./kgm.).....	10	1½–10	4.3

TABLE 2

Lactic acid, milligrams per cent in brain and blood of cats

CONDITION	LACTIC ACID, MGM. PER CENT		
	Brain		Blood
Control	23		
11% oxygen	24	32	23
	35	43	19
	47	48	
8% oxygen	25	32	24
	44	44	30
	48	59	
	62	84	

oxygen brain lactic acid values are still higher. The table presents typical results of a long series of experiments. The value for the controls, 23 mgm. per cent, is the average obtained on 10 animals. The averages for 11 per cent and 8 per cent oxygen are 38 mgm. per cent and 50 mgm. per cent respectively. The values for blood lactic acid are not significantly raised by the respiration of these gas mixtures.

Table 3 presents the effects on 3 of the 6 dogs exposed to the respiration of 8 per cent oxygen. In every instance the cerebral arterio-venous oxygen difference decreases and does so progressively with time. Three observations of the 5 made after the injection of iodoacetate reveal that the initial cerebral arterio-venous oxygen difference is increased after injection.

DISCUSSION. The results reveal that during partial anoxia the brain of the adult dog obtains sufficient energy from the anaerobic cleavage of carbohydrate to prolong significantly the survival period. When glycolysis is inhibited by the injection of iodoacetate in dogs respiring 8 per cent oxygen the survival period is shortened. Additional proof of the development of energy from carbohydrate during anoxia was obtained from a quantitative analysis of samples of brain and blood for lactic acid. In these animals the concentration of lactic acid in the brain was higher than in controls not subjected to anoxia. The cerebral lactic acid originated in the brain and did not diffuse to the brain from the blood because the concentration of lactic acid was higher in the cat brain than in the blood. The maintenance of the higher concentrations of lactic acid in the brain than in the blood may be accounted for by a certain degree of cerebral capillary impermeability to electronegative substances including lactic acid (6). The decrease of the cerebral arterio-venous oxygen differences in dogs respiring 8 per cent oxygen indicates that the respiration of this gas mixture does

TABLE 3

Arterio-venous oxygen difference after 8 per cent oxygen or iodoacetate

Volumes per cent

CONDITION	CONTROL	11 MINUTES	31 MINUTES	60 MINUTES
8% oxygen	11.3	7.3	5.8	
	6.9	4.3		2.6
	7.7		3.4	
Iodoacetate, 32 mgm./kgm.	6.8	8.7		
	6.8	9.3		
	7.0	10.2		

not furnish adequate supplies of oxygen to the brain. In every instance the cerebral arterio-venous oxygen difference diminishes during anoxia. The smaller arterio-venous oxygen differences are caused by a more rapid cerebral blood flow, a response to cerebral anoxia. In unpublished experiments on dogs anesthetized with Dial the respiration of 11 per cent oxygen had no effect on the cerebral arterio-venous oxygen differences. In cats anesthetized with chloralose, Courtice (7) observed that the cerebral arterio-venous oxygen difference did not begin to diminish until the respired oxygen fell to 15 per cent. The different sensitivities to anoxia exhibited with barbiturate and chloralose may be attributed to the fact that chloralose depresses the central nervous system and the respiratory centers less than do the barbiturates. Acceleration of circulation rate in response to cerebral anoxia may therefore develop with a lesser degree of oxygen deprivation under chloralose anesthesia.

The injection of iodoacetate increased the cerebral arterio-venous oxygen difference in dogs breathing room air. It is not likely that iodoacetate increased cerebral oxygen consumption but rather cerebral blood flow was slowed perhaps due to the effect of iodoacetate on the heart or capillaries. Irrespective of any

change in the rate of cerebral circulation, it should be noted that the oxygen intake continued despite a probable inhibition of cerebral glycolysis.

It is of some interest that after trial of various concentrations of iodoacetate a dose of 32 mgm. per kilo was chosen as the concentration compatible with adequate survival in air yet assuring rapid exitus while respiring 8 per cent oxygen. Henderson and Greenberg (8) have previously determined that iodoacetate in doses of 33 mgm. per kilo prevented the accumulation of lactic acid in the blood despite prolonged anoxia.

The brain probably employs more than one path for the oxidation of carbohydrate in the formation of energy. By one path, designated as Path I, glucose breaks down to pyruvic acid before oxidation to carbon dioxide and water, the Embden-Meyerhof scheme. But when this path is blocked by iodoacetate, the oxidation to carbohydrate continues by another path, Path II. There are, therefore, at least 2 paths for the oxidation of carbohydrate. On the other hand, during anaerobiosis, the brain can obtain energy only by the first path and the formation of lactic acid. The injection of iodoacetate during anaerobiosis therefore robs the brain of its only remaining source of energy; the formation of lactic acid by Path I is inhibited and cerebral function ceases. Both aerobic and anaerobic production of cerebral energy is greater in the adult than in the infant brain (1, 2, 9). The increase in the aerobic processes, however, is larger than in the anaerobic. As a result of this shift to aerobic processes the adult is less tolerant to anoxia than is the infant.

SUMMARY

A study was made in order to evaluate the significance of the anaerobic development of energy from the cleavage of carbohydrate for the maintenance of cerebral function. 1. In dogs anesthetized with pentobarbital and respiring 8 per cent oxygen in nitrogen the cerebral arterio-venous oxygen differences decrease indicating an inadequate supply of oxygen to the brain. 2. Lactic acid accumulates in the brain of cats anesthetized with pentobarbital and subjected to the respiration of 8 per cent oxygen, as the cleavage of carbohydrate affords energy for the maintenance of cerebral functions during anoxia. 3. The injection of iodoacetate, 32 mgm. per kilo, which inhibits the cleavage of carbohydrate shortens the survival periods of dogs anesthetized with pentobarbital and respiring 8 per cent oxygen. It may, therefore, be concluded that during a period of partial anaerobiosis the brain of the adult dog or cat obtains some of the energy required for its maintenance by the anaerobic splitting of carbohydrate and the formation of lactic acid. It should be pointed out however that the ability of the adult to withstand anoxia is much more limited than the infant.

REFERENCES

(1) FAZEKAS, J. F., F. A. D. ALEXANDER AND H. E. HIMWICH. This Journal **134**: 281, 1941.
(2) HIMWICH, H. E., A. O. BERNSTEIN, H. HERRLICH, A. CHESLER AND J. F. FAZEKAS. This Journal **135**: 387, 1942. .

(3) FAZEKAS, J. F. AND H. E. HIMWICH.　J. Biol. Chem. **139**: 971, 1941.

(4) FRIEDEMANN, T. E., M. COTONIO AND P. A. SHAFFER.　J. Biol. Chem. **73**: 335, 1927.

(5) VAN SLYKE, D. D. AND J. M. NEILL.　J. Biol. Chem. **51**: 523, 1924.

(6) FRIEDMANN, U.　Physiol. Rev. **22**: 125, 1942.

(7) COURTICE, F. C.　J. Physiol. **100**: 198, 1941–42.

(8) HENDERSON, Y. AND L. A. GREENBERG.　This Journal **107**: 37, 1934.

(9) TYLER, D. B. AND A. VAN HARREVELD.　*This Journal* **136**: 600, 1942.

ESERINE, ACETYLCHOLINE, ATROPINE AND NERVOUS INTEGRATION

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According to the "humoro-electrical" theory, activation of nerve cells transpires in two stages—humoral and electrical (Gesell, Brassfield and Hansen, 1942). Impulses impinging on the nerve cell release the neurohumor. Acetylcholine by virtue of its rate of destruction pools at the dendrites and cell body and provides an adjustable electrochemical voltage, the intensity of which is determined by the size of the pool. A resulting continuous current leaves the neuron at the axon hillock and fires the neuraxon at a frequency proportional to its intensity. Artificially administered extrinsic acetylcholine on reaching nerve cells, adds to the existing intrinsic acetylcholine and potentiates the reflexes motivated by the existing physiological bombardment. Eserine protects the intrinsic acetylcholine liberated by physiological bombardment and produces similar potentiation of physiologically motivated reflexes (Gesell, Hansen and Worzniak, 1943). Atropine by counteracting the effects of acetylcholine exercises a depotentiating influence. Because the effects of acetylcholine, eserine and atropine are necessarily linked with prevailing synaptic bombardment, they offer unusual promise as chemical tools in the unravelling of the mechanisms of nervous integrations.

PROCEDURES. Our methods are described in part in earlier studies on humoral intermediation of nerve cell activation (Gesell, Hansen and Worzniak, 1943). In the present observations (all on the dog) the abdominal and costal respiratory movements and the contractions of the accessory respiratory muscles of the face were recorded. This was essential to show qualitative changes in breathing which ordinarily fail to reveal themselves in the spirometer record alone. The thoracic and abdominal movements (change of girth) were recorded with a modification of the torsal band method (Gesell and Moyer, 1935). The facial muscular contractions were registered with a vertical writing point attached to the fold of skin at the angle of the mouth. Downstroke represents inspiration in the band records and expiration in the facial tracings unless otherwise specified. Facial, thoracic, abdominal and spirometer records are labelled F, T, A and S.

RESULTS. 1. *Respiratory response to eserine conforming with theoretical expectations.* Granting that breathing is synaptically motivated, eserine, by virtue of its anticholinesterase activity might be expected to produce the same effects as acid, namely, a co-ordinated hyperpnea. But eserine produces two types of response illustrated in figure 4—a simple hyperpnea such as may be expected from excess of CO_2 and an unanticipated deviation which differs in striking and characteristic ways to be described below. Of the two changes the expected

result seen in figure 1 is by far the less common.   This observation was made during pneumothorax plus constant ventilation to assure a uniform pH.   It compares in essentials with the response to simple asphyxia during pneumothorax minus ventilation to assure a diminishing pH illustrated in figure 2.   The records from above down are facial, chest and abdominal movements.   The slowness of the development of hyperpnea during eserine poisoning and the protracted recovery (not shown) seem merely to reflect a very gradual accumu-
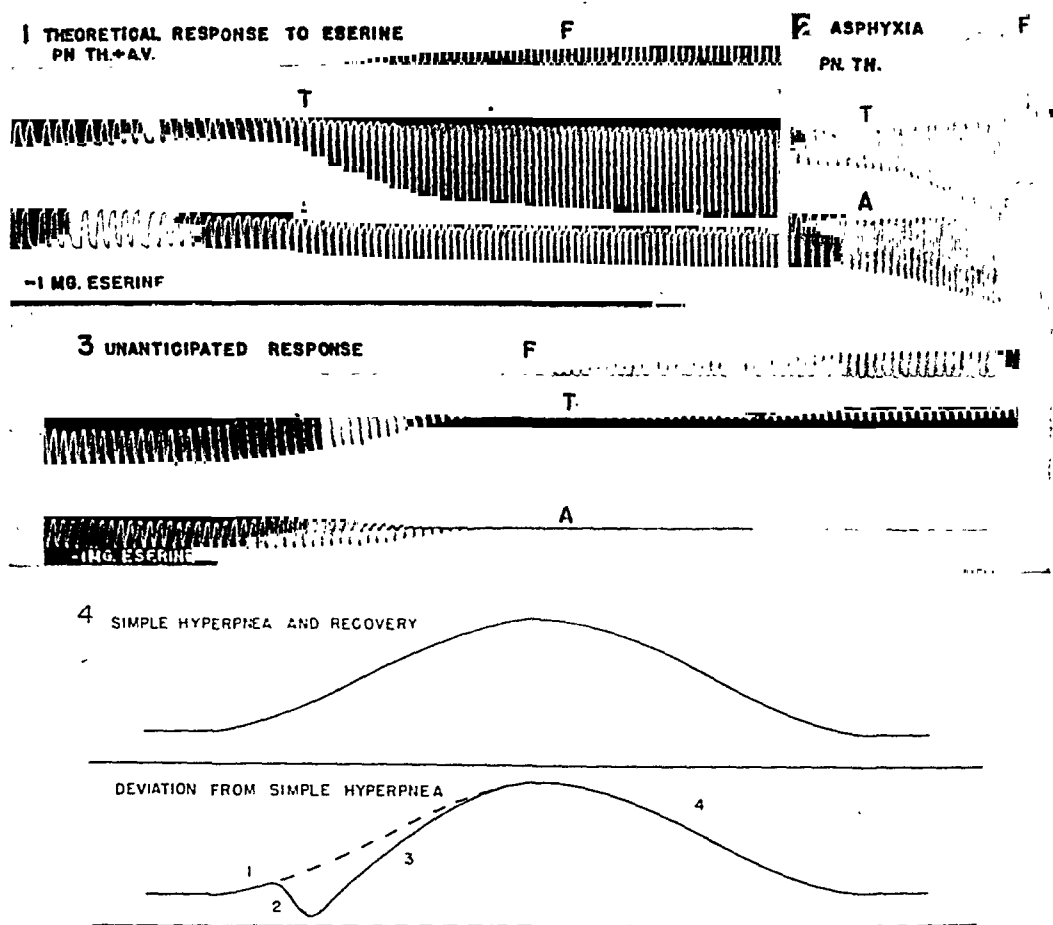
Figs. 1–4

lation and disappearance of acetylcholine.   Return to normal breathing may require 1 to 3 hours.

2. *Quantitative deviations from the theoretical response to eserine.*   Deviation from the expected response to eserine (see schema, fig. 4) consists of an early interruption of the initial hyperpnea which divides the complete response into four instead of two phases: 1, a relatively brief hyperpnea; 2, a subsequent diminishing ventilation; 3, a relatively prolonged period of increasing ventilation, and 4, a protracted return to normal.   See spirometer tracing of figure 6 for stages 1, 2 and 3.   Stage 4 is omitted.

Abolition of pulmonary ventilation in stage 2 (see figs. 10 and 11) would indicate a complete paralysis of the respiratory center.   The sudden appearance

of powerful sporadic inspirations scattered among the periods of apnea or during shallow breathing in that same stage in figures 5, 6 and 7 however speak for a highly excitatory condition of the respiratory center. In a like manner the diminution of tidal air in figure 11 agrees with a depression of the center, but the concurrent accelerated frequency suggests the reverse. The final apnea, therefore, cannot be interpreted as an unquestionable paralysis. The significance of the changes in pulmonary ventilation is more effectively approached by a consideration of the qualitative changes in breathing.

3. *Qualitative changes in breathing produced by eserine.* The qualitative changes which eserine produced upon the respiratory act proved far more significant than the quantitative fluctuations in pulmonary ventilation because they revealed highly significant differential effects of nervous imbalance. At the height of eserine poisoning breathing is radically changed. The accessory respiratory muscles of the face and neck contract vigorously, as if the animal were suffering from intense asphyxia, while at the same time the torso shows no respiratory movements whatever. The condition develops slowly. As one muscular activity diminishes another increases with the resultant effect that respiration changes from a normal and predominantly inspiratory type to a predominantly expiratory type. Eventually torsal respiratory movements disappear while facial inspiratory contractions persist at maximum presenting a different type of inspiratory breathing from that of eupnea. With recovery these events are reversed. Facial, chest and abdominal movements as well as pulmonary ventilation were therefore recorded. Figures 5, 6, 7, 14, 15, and others illustrate some of the outstanding points in this extraordinary phenomenon.

Figure 6 is our most complete illustration, lacking only the last stages of an uneventful recovery back to eupnea. Two milligrams of eserine were injected intravenously at A'. Following the initial hyperpnea at B thoracic and abdominal movements and pulmonary ventilation diminish after which breathing becomes decidedly irregular in both costal and abdominal segments. Irregularity of breathing is ushered in by a progressive constriction of the chest. At maximum constriction normal respiratory rhythm in the abdominal segment is abolished. Only occasional sporadic inspirations break through. In the chest however respiratory contractions continue without interruption and increase in amplitude at an early stage of poisoning. Normal respiratory rhythm in the abdominal segment returns only after the chest has begun to relax. Abdominal movements then increase in strength along with the chest. Irregularity of breathing disappears as the expiratory activity of the chest weakens.

Dominance of costal breathing results from at least two changes in muscular activity: 1, a weakening of diaphragmatic contractions, and 2, a strengthening of costal expiratory contractions. Of the acquired qualitative changes in breathing, costal expiratory activity appears first and facial inspiratory contractions appear second, after an interval of about 3.5 minutes. Both activities increase in intensity for another 4 minutes up to point C. This marks the peak of eserine poisoning beyond which facial inspiratory and costal expiratory activity diminish. Facial contractions disappear within 9 minutes, at point D.

Costal expiratory activity is still present at the end of the record. The order of appearance of costal expiratory and facial inspiratory activity and the reversed order of diminution and disappearance of these activities is rather characteristic.

. Several features of this response seem exceptionally pertinent. *Inspiratory* contractions of the diaphragm *weaken* as the *expiratory* contractions of the chest *increase*. *Inspiratory* contractions of the diaphragm *weaken* as *inspiratory* contractions of the face *increase*. Neither combination has the earmarks of co-ordination toward attainment of hyperpnea. On the other hand the simultaneous increase of inspiratory and expiratory activity in the chest is indicative of a localized co-ordination. Failure of the diaphragm seems to be the major disco-ordination.

Figure 14 shows an exceptionally orderly series of events in the three major alterations in a relatively slowly breathing animal. Irregularity of breathing is missing despite intact vagal reflex paths. Diaphragmatic contractions diminish and facial contractions increase. After an initial decrease the thoracic contractions increase along with the facial contractions. The increase in thoracic activity is primarily expiratory. As noted in figure 6, there are opposing and co-ordinated changes in activity in three groups of muscles. There is an increasing expiratory activity in the chest, and an increasing inspiratory activity in the face associated with a decreasing inspiratory activity in the diaphragm. Deficient diaphragmatic movement is compensated in part by increasing costal movement, mainly expiratory. Tidal air increases after an initial depression despite abdominal inactivity. Breathing changes from a predominantly inspiratory type toward an increasingly expiratory type. Ten milligrams of atropine injected into the vertebral artery brings on a sudden reversal of effects differing from ordinary recovery mainly in the abruptness of the changes. Facial contractions cease, active expiratory contractions disappear (note the sudden drop in the costal expiratory level), diaphragmatic contractions suddenly return and the animal is back to the inspiratory type of breathing.

In general the spirometer records tell little about the qualitative changes in breathing but on rare occasions they differentiate in a single tracing the active and passive phases of inspiration and of expiration. Such was the fortunate case in figure 15. The dog was vagotomized and breathing was relatively slow. During eupnea inspiration is certainly active and expiration most probably passive—at least predominantly passive. This dominance of inspiratory activity persists throughout the initial hyperpnea, A, and the following hypopnea, B, up to point C. After point C the respiratory cycle divides itself into five distinct phases: 1, active inspiration; 2, passive expiration; 3, inactive expiratory pause; 4, active expiration; 5, passive inspiration. The quiescence of the torso during the expiratory pause produces a series of notches and a resulting demarcation line which reveals the relative intensities of contraction of the inspiratory and expiratory muscles. After the sudden reduction of respiration up to point C ventilation increases up to point E where the effects of eserine are interrupted by an intravenous injection of atropine. From C to D ventilation increases

despite a steadily weakening inspiration. The adjustment is made by a steadily increasing expiration. From D to E breathing continues to increase despite a slowly diminishing expiratory activity. The adjustment now comes through an increasing strength of inspiratory contraction. A sudden and striking transformation of breathing occurs at E comparable to a rapid and condensed recovery. Thus frequency of breathing diminishes. The expiratory component disappears. Accessory inspirations vanish. Diaphragmatic contractions return in force. Breathing is back to the predominantly inspiratory type.

What do these changes signify, individually and collectively? The increasing and decreasing force of facial contractions are a perfect duplication of hypercapnic hyperpnea and recovery. They agree with a progressively increasing and decreasing pooling of acetylcholine in the facial accessory arcs. Increasing and decreasing intensity of thoracic expiratory contractions agree with an increasing and decreasing pooling at the corresponding expiratory neurones. It would be stretching the point to attribute the irregularity of torsal inspiration noted in figure 6 to a coinciding irregularity of pooling in the torsal inspiratory arcs. There is no evidence that eserine, by itself, is capable of such effects. It would seem more logical to attribute the irregularity of breathing to sporadic changes of imbalance between the opposing half-centers and to accept the stronger contractions as the more reliable measure of the potential activity of the torsal inspiratory neurones. Working on that assumption we have noticed that the inspiratory contractions tend to divide into two groups, weaker contractions which decrease in intensity up to the peak of eserine poisoning and greater sporadic contractions which tend to increase in power as eserine poisoning increases (see figs. 5, 6 and 13). How are these diametrically opposite changes to be reconciled? Excessive expiratory activity could impress excessive inhibition on the inspiratory neurones via reciprocating connections. On the other hand an increased pooling of acetylcholine at the inspiratory neurone could account for the increasing intensity of those inspirations which sporadically break out of the imbalance (see Gesell and Hamilton, 1941, and review by Gesell, 1940). Were it not for the disco-ordinate potentiation of the expiratory neurones eserine hyperpnea might be as highly co-ordinated as hypercapnic hyperpnea.

While the increase of the larger inspiration does not always follow a smooth curve, such as illustrated in figure 13 the general tendency in that direction is unmistakable. On the whole smaller injections of eserine are more likely to produce irregularity of breathing and an increase and decrease of depth of the two groups of inspirations. Compare figures 5, 6 and 7 in which 1.4, 2.0 and 3 mgm. of eserine were injected. Large injections tend to prevent the appearance of the powerful inspirations as in figure 12 or to delay their onset as in figure 7.

4. *A possible rôle of bronchiole constriction in the respiratory response to eserine.* It has been suggested to us that a constriction of the bronchioles resulting from accumulation of acetylcholine at the pulmonary motor endings might impede the movement of air in and out of the lungs, at the same time allowing unimpeded contractions of the facial muscles which are not exposed to this mechan-

ical resistance. Two observations question the importance of bronchiole constriction—absence of powerful torsal respiratory efforts in severe eserine poisoning
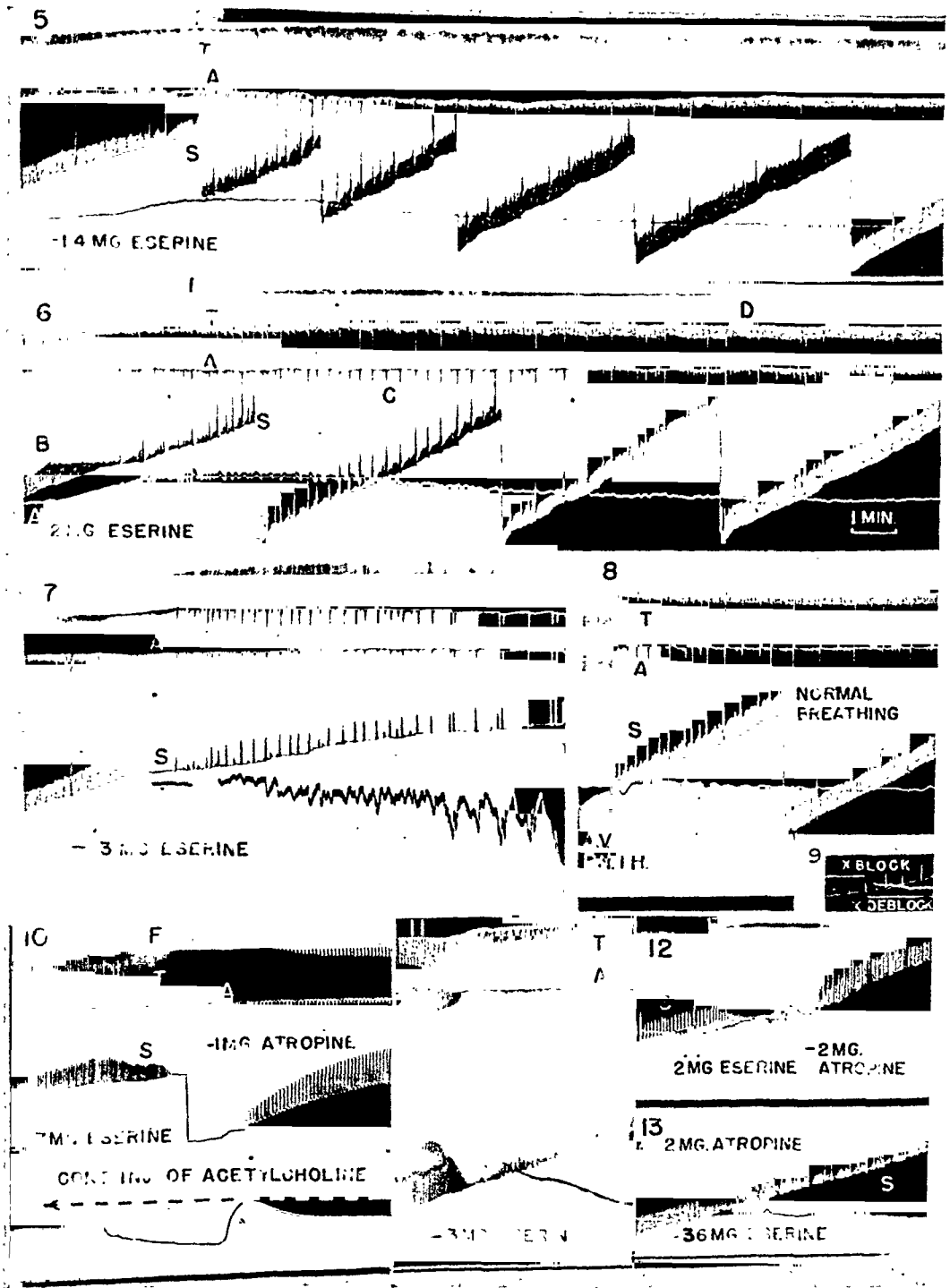
Figs. 5–13

such as are ordinarily seen in all forms of mechanical asphyxia (pneumothorax, fig. 2, or clamping of the trachea) and the actual occurrence of deep breaths interspersed among the subnormal inspirations and apneas (figs. 5, 6 and 7).

Nevertheless it seemed desirable to seek more quantitative evidence by recording the effects of eserine during constant artificial ventilation administered during double open pneumothorax. Constant pulmonary ventilation has the purpose of eliminating acidity as a modifying factor and double pneumothorax assures freedom of movement to all groups of respiratory muscles. Should bronchiole constriction occur it can effect neither the ventilation of the blood, nor the extent of the contraction of the respiratory muscles. The customary hyperpneic activity of the facial accessory muscles and the simultaneous torsal apnea (fig. 3) show that the peculiar respiratory response to eserine must be sought in some detail other than bronchial constriction.

5. *Effects of size of injection of eserine.* The amount of eserine injected influences the respiratory response in several ways (see figs. 5, 6 and 7). One and four-tenths milligrams of eserine evoked no facial accessory response, 2 mgm. evoked a medium response and 3 mgm. a powerful response. The response appeared within approximately 5 minutes after the injection of 2 mgm. and within approximately 1.5 minutes after 3 mgm. Irregularity of torsal breathing and regularity of facial breathing occurred with all doses. The appearance of the sporadic deep breaths as mentioned above was momentarily delayed by the large injection. Normal respiratory rhythm persisted in both costal and abdominal segments after the smallest injection. With the intermediate dose of 2 mgm. the chest followed facial contraction consistently while the abdomen followed only sporadically. With the large injection the normal rhythm disappeared in both torsal segments, presenting an interesting picture of two simultaneous respiratory rhythms, a rapid normal rhythm in the upper facial arcs and a slow or partially blocked rhythm in the lower segments.

The earlier appearance of accessory contractions with large injections of eserine indicates a more rapid accumulation of acetylcholine at the facial neurones under the increased protection offered by those doses. Presence of torsal response and absence of facial response to the smallest injection (1.4 mgm.) indicates lighter bombardment of the facial arcs, i.e., a smaller release of acetylcholine requiring greater potentiation to bring it to threshold stimulation. More extensive elimination of torsal inspiratory movements with increasing doses of eserine indicates an increasingly disproportionate potentiation of the expiratory neurones and a correspondingly greater domination of the inspiratory neurones (see below).

6. *The factor of asymmetrical bombardment and asymmetrical potentiation of opposing half-centers.* Pronounced asymmetrical activity of the opposing half-centers during ordinary eupnea suggests a correspondingly pronounced asymmetry of bombardment. The successive order of appearance of inspiratory, expiratory and accessory contractions during asphyxia following upon artificially induced hypocapnic apnea would seem to reflect the relative intensities of the corresponding bombardments. Increasing anticholinesterase activity (hypercapnia in this instance) superimposed upon apnea must theoretically potentiate all existing bombardments and call additional respiratory muscles into play in the order named. Should the acetylcholine machinery of integration be sus-

ceptible to disco-ordinate (i.e., disproportionate) potentiation, let us say of the more lightly bombarded expiratory neurones, the inspiratory neurones must give way in part to the dominance of the superactivated expiratory neurones via reciprocal inhibition.

The existence of such a mechanism is actually suggested in the response of muscle to indirect stimulation of low and high frequency (Rosenblueth and Morrison, 1937). Eserine injected during the course of such stimulation was found to potentiate the effects of infrequent stimulation (explained by moderate accumulation of acetylcholine at motor end plates) and to paralyze the muscle to frequent stimulation (explained by excessive accumulation of acetylcholine). Preliminary experiments on the diaphragm agree with those of Rosenblueth and

Figs. 14–15

Morrison. We have reversed the procedure and sampled the response of muscle to electrical stimulation during the course of eserine poisoning. To mention a single example—the diaphragm was found to respond with a single twitch to a continuing stimulation of the phrenic nerve at a frequency of 45 per second and with a series of twitches to a stimulation frequency of 15 per second. Small and large concentrations of acetylcholine exercise comparable potentiating and paralyzing effects upon sympathetic and parasympathetic ganglia.

Since our findings on respiration could be explained by peripheral muscular as well as by central nervous action it was desirable to inquire into the problem of relative susceptibility of muscle and center and thus establish the main site of action of eserine.

7. *The site of disco-ordination of the respiratory act in eserine poisoning.*

Eserine unquestionably produces a central disco-ordination because the intensity of the electrical potentials of the phrenic nerve and the intensity of diaphragmatic contraction run parallel courses. When contractions cease electrical activity is missing and when contractions return and increase in volume the same changes in phrenic electrical activity occurs. The fact that the diaphragm will respond to artificial stimulation of the phrenic nerve at a time when physiological contractions and phrenic potentials are missing indicates that central integration is more sensitive to eserine than is simple intermediation at the motor end plate of the diaphragm. With larger injections however both muscle and center may be involved.

Parallel changes of expiratory nerve potentials and expiratory muscle activity give double assurance that the centers are importantly involved in the respiratory response to eserine poisoning.
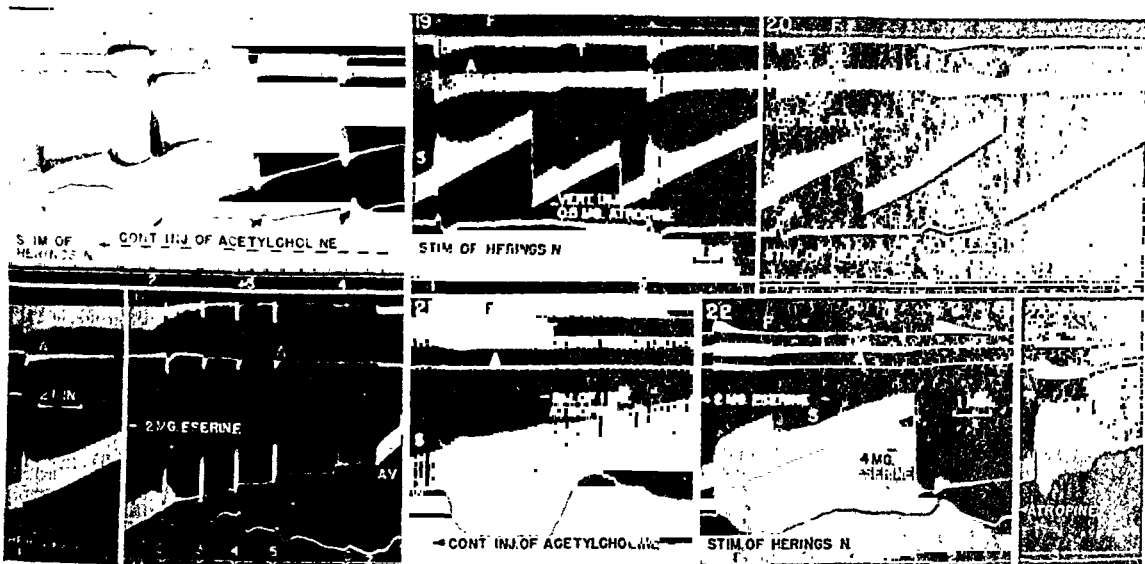
Figs. 16-23

Absence of phrenic potentials during eserine hypopnea however must not be interpreted as *paralysis* of the inspiratory half-center unless reciprocal inhibition is eliminated. Evidence on that issue must be sought in other ways.

8. *Central paralysis of the inspiratory neurones versus reciprocal inhibition via excitation of expiratory neurones.* Inhibition and paralysis differ in that one implies a functional state and the other a loss of function. Reflex stimulation of the respiratory center should allow experimental differentiation. A paralyzed inspiratory half-center should be incapable of responding to an inspiratory stimulation (Hering's nerve) whereas an inhibited center should yield (see figs. 17 and 18). Although eserine had virtually arrested breathing in figure 18 stimulation of Hering's nerve produced a more powerful response than before. Breathing bursts out of apnea with a surprising suddenness and frequency and ceases without a sign of after-discharge. Only two breaths occurred during stimulation before eserine was injected as compared with 6 breaths during eserine poisoning.

The suddenness with which breathing stops at the end of stimulation is a most interesting phenomenon because eserine is generally regarded as favoring after-discharge. Fulton (1938) believes that after-discharge is highly susceptible to inhibition. Can it be that the excessive expiratory activity, as displayed in the chest, is reciprocally opposing the tendency of the inspiratory half-center to after-discharge? If so the "apnea" which obtains between stimulations is an indirect expression of excitation rather than a direct expression of paralysis, i.e., an indirect inhibition resulting from excessive and imbalanced stimulation of the expiratory neurones.

The extremely rapid breathing which stimulation of Hering's nerve produced may also be related to the dominant expiratory potentiation by eserine poisoning because stimulation of the predominantly expiratory excitatory vagus is known to intensify and prolong the expiratory phase of breathing thereby retarding rhythm; and stimulation of the predominantly inspiratory excitatory Hering's nerve (vagi cut) is known to intensify and prolong the inspiratory phase of breathing and thus retard rhythm. Simultaneous stimulation however does not produce an additive slowing. Instead, both phases of breathing are shortened and frequency increased. It is believed that stimulation of Hering's nerve in figure 18 adds a powerful inspiratory drive to a powerful expiratory drive already prevailing during eserine poisoning. In so doing it corrects a respiratory imbalance and forces the center to discharge at an accelerated rhythm.

Although a predominant potentiation of the expiratory neurones is sufficient reason for apnea, excessive eserine poisoning is capable of producing direct paralysis of breathing as well. This is seen in figure 18 where the potentiation, evident in the first four stimulations, suddenly gives way to paralysis as the intensity of poisoning advances (stimulations 5 and 6). Note the fall in blood pressure.

9. *Proprioceptive reflexes in eserine poisoning.* The central effects of acetylcholine and eserine are importantly modified by prevailing physiological reflexogenic support (Gesell, Hansen and Worzniak, 1943). Vagal proprioceptive reflexes are especially effective in molding the patterns of breathing during eserine poisoning. This is evident in the irregularity of breathing (figs. 5, 6 and 7), and the readiness by which irregularity is abolished by vagotomy or vagal block (fig. 9). Subsequent X deblocking re-establishes the irregularity. This influence of the vagus nerves seems to originate in the stretch reflex rather than the collapse reflex for if the lungs are maintained at subnormal volumes (artificial ventilation during pneumothorax, vagi intact) breathing continues with perfect regularity (fig. 8). If the lungs are now brought back to normal volume (discontinuance of artificial ventilation and pneumothorax) irregularity of breathing returns immediately.

Sustained pulmonary inflation to supernormal volume and simple eserine poisoning show analogous effects pertinent to our problem (Gesell, 1940). Both establish a dominating expiratory activity. Both hold inspiration in abeyance and create apneic pauses of varying duration. These pauses are interrupted by irregular breaths of varying depths, normal, subnormal, and supernormal.

Each breath transpires with accelerated velocity, expiration often retracing the inspiratory stroke. Presumably these analogous effects have similar integrations.

The dual excitatory mechanisms of vagal function hold that a stretched condition of lungs reflexly releases an increased amount of acetylcholine at both half-centers. Because more acetylcholine is released at the expiratory half-center, the inspiratory half-center is held in reciprocal abeyance. When, however, the inspiratory half-center breaks out of the dominance of the expiratory half-center it discharges violently because the superactivity of the inspiratory excitatory fibers has accumulated excessive amounts of acetylcholine about the inspiratory neurones.

Theoretically analogous conditions prevail in simple eserine poisoning. Acetylcholine accumulates in the inspiratory and expiratory neurones thereby increasing the potential power of every impulse impinging on both groups of cells. Disproportionate intensification of expiratory activity holds the inspiratory neurones in abeyance. When however they do discharge they do so under the driving force of excessive acetylcholine. Inspiration is therefore rapid and powerful. This produces a rapid inflation of the lungs and powerful reflexogenic release of acetylcholine at the inspiratory neurones which in turn is spared by the anticholinesterase activity of eserine. Inspiration gets out of control and reaches supernormal depths. The simultaneously intensified expiratory stretch reflex and the greater accumulation of acetylcholine at expiratory neurones intensifies the expiration which follows.

10. *A comparison of the effects of eserine and of acetylcholine.* Granted that eserine produces a gradual accumulation of acetylcholine at nerve cells, a slow and continuous injection of a weak solution of acetylcholine might be expected to produce approximately similar conditions. Acetylcholine was injected with a specially devised pump into the carotid artery (2 cc. per minute of 1/10000 solution) after having denervated the carotid bodies (fig. 16). The period of injection is indicated. The effects are remarkably similar to those of eserine in figure 18. There is a temporary increase of tidal air, a marked increase of frequency of breathing with a decided falling off of tidal air. The chest shows marked expiratory constriction, the abdomen passive dilatation and the chest movements are better conserved than the abdominal. There is the same augmented increase of respiratory response to faradic stimulation of Hering's nerve. Reflexogenic ventilation is trebled.[1]

11. *Antagonism between atropine, and acetylcholine or eserine.* The effects of atropine upon breathing during eserine poisoning are exceptionally striking. Respiration which has been completely stopped by excessive eserine poisoning is restored within a few seconds by small amounts of atropine (approximately 1 mgm.). When administered during milder poisoning atropine reverses each qualitative change in breathing which eserine had set up (see figs. 14 and 15).

[1] Schweitzer and Wright state that "The excitatory action of eserine cannot be due to its anticholinesterase action as it is the converse of the action of acetylcholine" (J. P. 89: 196, 1937).

The highly active accessory respiratory muscles of the face stop contracting, the active expiratory contractions of the chest disappear, and the inactive or weakened diaphragm contracts with greater vigor. Similar antagonism exists between atropine and acetylcholine. When acetylcholine is injected continuously in dilute solution to simulate the conditions of eserine poisoning atropine restores torsal pulmonary ventilation if breathing has been suppressed (fig. 10). On the other hand if breathing has been increased, atropine diminishes pulmonary ventilation (fig. 21), agreeing with the findings of Miller, Stavraky and Woonton (1940) on the cerebral cortex.

This opposing action of atropine upon extrinsic acetylcholine is in agreement with the well recognized antagonism of the muscarine effects of acetylcholine and those of atropine in the parasympathetic system. Since it is highly probable that eserine increases the amount of extra-neurocellular acetylcholine the action of atropine during eserine poisoning may be analogous to that during extrinsic acetylcholine poisoning.

Theoretically atropine could produce significant effects at several points, at the myoneural junction, at synaptic junctions in the chemoceptor organs (v. Euler, Liljestrand and Zotterman, 1941) and in the centers. The elimination of the myoneural junction as a factor in the production of the typical respiratory response to moderate amounts of eserine eliminates this site of action of atropine in the reversal of eserine effects. This conclusion is also supported by our direct observations on nerve and muscle action potentials. When atropine is injected directly into the vertebral artery during eserine poisoning (fig. 14) the effects are more profound, and more sudden than when injected intravenously (fig. 15). The restoration of breathing within a period of two to three seconds indicates a central action of the drug. Comparable effects of atropine during eserine poisoning after denervation of the carotid bodies and double vagotomy supports this conclusion (not illustrated).

We are forced to believe, contrary to generally accepted opinion, that atropine is capable of exercising a powerful anti-acetylcholine and anti-eserine action in the nerve cells of the central nervous system. Our findings and those of Miller, Stavraky and Woonton (1940) corroborate those of Marrazzi (1939) in peripheral ganglia and make the observation general for all nerve cells.

12. *Central effects of atropine upon eupneic breathing and on the reflex response to faradic stimulation of Hering's nerve.* Small injections of atropine into the vertebral artery after denervation of the carotid bodies and double vagotomy diminishes breathing appreciably. In figure 19 eupnea was reduced approximately 20 per cent by 0.5 mgm. and artificial reflexogenic hyperpnea, produced by faradic stimulation of Hering's nerve, was diminished a comparable amount. Compare the pre- with the post-atropine response. Proper preconditioning with eserine will reverse the effects of atropine upon reflexogenic response (figs. 22 and 23). The first stimulation of Hering's nerve, administered about 15 minutes after an intravenous injection of 2 mgm. of eserine, showed a marked potentiation of the facial, costal and abdominal movements (control omitted). Four additional milligrams of eserine after stimulation no. 1 abolished chest and abdominal movements and introduced a series of minute facial contractions. Stimulation

no. 2 applied during this increased anticholinesterase influence produced a relatively smaller response of the chest and abdomen and a relatively larger response of the facial accessory movements than stimulation no. 1. This response is reversed by atropine in stimulation no. 3. Torsal movements are now increased more than they were in stimulation no. 2 and facial response is relatively diminished. According to our reasoning acetylcholine exceeded the limits of potentiation at the heavily bombarded torsal arcs in stimulation no. 2 but remained within the limits of potentiation at the more lightly bombarded facial arcs. Elevation of the threshold of stimulation by atropine abolished the paralytic action of high concentration of acetylcholine at the heavily bombarded arcs and kept the effects of the acetylcholine within the limits of potentiation. But at the facial arcs where acetylcholine had failed to reach paralytic concentrations in stimulation no. 2, atropine raised the threshold excessively and thereby weakened the stimulating effects of acetylcholine released during stimulation no. 3.

The simultaneous occurrence of both excitatory and depressing effects at individual functional units of the respiratory center from a single injection of atropine is interesting in view of the fact that central effects of both types are ascribed to atropine (Goodman and Gilman, 1941).

13. *The effects of preconditioning with atropine upon the respiratory response to eserine.* Atropine in quantities as minute as 1 mgm. raises the threshold of respiratory response to eserine enormously. Often the effects are mainly quantitative, at other times marked qualitative effects occur as well. Instead of the typical four phasic response eserine may produce a simple hyperpnea comparable to that of hypercapnia (fig. 20). A more perfect balance between half-centers is in some way attained. If this better balance is attributable solely to an increase of threshold it illustrates another simple means by which nervous integration can be strikingly modified.

COMMENTS. The great value of eserine as a physiological tool lies in its accepted mode of action. Though non-stimulating in itself its end result is stimulation. Like the hydraulic dam which pools the energy of a flowing stream of water and thereby motivates hydraulic machinery, eserine pools the nervous power of a continuing stream of nerve impulses. Without primary disturbance in the volume of physiological bombardment it raises the level of acetylcholine and supermotivates the nervous machinery to which this humoral energy is hitched. Faced with this peculiar action it seems increasingly difficult to deny the steady or "tonic" nature of nervous forces (Gesell, 1940) or to defend a purely electrical and temporally rigid concept of synaptic "transmission".

It stands to reason that a powerful neuro-humoral drive calls for a proportionately powerful inhibition if one half-center is to give way to the other in rhythmical alternate activity. A unihumoral mechanism of nervous integration might conceivably meet such requirements on the assumption that acetylcholine is capable of producing either stimulation or inhibition depending upon where it is released—excitation if released at the dendrites and inhibition if released at some strategical counterelectromotive position such as the axon hillock or base of dendrite. Without disrupting the machinery of integration a

simultaneously increased anticholinesterase action at both excitatory and inhibitory dendrites might produce a co-ordinated hyperactivity such as is seen in hypercapnic hyperpnea. A dual humoral system of nervous integration based on the experiments of Marrazzi (1939) on autonomic ganglia is an alternate possibility.

SUMMARY

Among the outstanding observations on eserine were 1, a reinforcement of thoracic expiratory contractions; 2, a temporary irregularity of strength of torsal inspiratory contractions associated with subnormal pulmonary ventilation; 3, an initiation and strengthening of the facial accessory respiratory contractions.

These effects occur with vertebral injections after chemoceptor denervation and are therefore central.

Irregularity of breathing consisted of shallow breaths tending to weaken and of sporadically deep breaths tending to strengthen as eserine poisoning increased.

Large injections intensified all effects of eserine including the curtailment of the more shallow breaths. Complete curtailment left the powerful inspirations separated by apneas of variable durations.

Corresponding apneas were absent in the facial rhythm. Respiratory block is indicated.

Increasing intensity of costal expiratory, of torsal inspiratory and of facial accessory inspiratory contractions are thought to indicate a progressive pooling of acetylcholine at all nerve stations where bombardment and release of acetylcholine occurs.

Failure of the smaller inspirations to attain maximum intensity is attributed to a disproportionate potentiation of expiratory neurones structurally coupled with their opposing inspiratory neurones. Only those inspirations breaking free of the dominating reciprocal inhibition are thought to gauge the potential activity of the inspiratory cells.

The effects of eserine were duplicated with slowly injected extrinsic acetylcholine.

Atropine produced profound central effects: 1, reversal of the response to eserine and extrinsic acetylcholine, and 2, diminution of breathing motivated by intrinsic acetylcholine (fig. 19) (eupnea and artificial reflexogenic hyperpnea). Antagonism was greater to extrinsic than to intrinsic acetylcholine.

It is thought that the support which our experiments give to the "humoro-electrical" theory of nerve cell function may prove basic to neurophysiological concepts.[2]

[2] Since our paper has gone to press highly significant data have appeared on synaptic and motor and plate potentials. Repetitive stimulation of preganglionic fibers (low frequency) produces individually independent synaptic potentials. With greater frequencies there is a stepped rise to a wavy plateau. This plateau is attained earlier and is higher the higher the frequency. Undulations corresponding to stimulation may disappear entirely. (See Eccles, J. P. 1943, 101, 465.) Such smoothed synaptic potentials would seem to correspond with the requirement of our concepts that pooling of acetylcholine provides an adjustable continuous current for activating the neuraxon as it leaves the axon hillock (see p. 383).

REFERENCES

GESELL, R., C. R. BRASSFIELD AND E. T. HANSEN. Fed. Proc. 1: 29, 1942.

GESELL, R., E. T. HANSEN AND J. J. WORZNIAK. This Journal, in press.

GESELL, R. AND C. MOYER. Quart. J. Exper. Physiol. 24: 315, 1935.

GESELL, R. AND M. A. HAMILTON. This Journal 133: 694, 1941.

GESELL, R. Ergebn. der Physiol. 43: 477, 1940.

ROSENBLUETH, A. AND R. S. MORISON. This Journal 119: 236, 1937.

FULTON, J. F. Physiology of the nervous system. Oxford Univ. Press, 1938.

MILLER, F. R., G. W. STAVRAKY AND G. A. WOONTON. J. Neurophysiol. 3: 131, 1940.

V. EULER, U. S., G. LILJESTRAND AND Y. ZOTTERMAN. Acta Physiol. Scandinav. 2: 1, 1941.

MARRAZZI, A. S. J. Pharmacol. and Exper. Therap. 65: 18, 1939.

GOODMAN, L. AND A. GILMAN. The pharmacological basis of therapeutics, MacMillan Co., New York, 1941.

MARRAZZI, A. S. This Journal 127: 738, 1939.

THE OCCURRENCE OF A VASOCONSTRICTOR SUBSTANCE IN BLOOD DURING SHOCK INDUCED BY TRAUMA, HEMORRHAGE AND BURNS

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Shock elicited by the tourniquet method and by hemorrhage is associated with arterial and arteriolar vasoconstriction until death of the animal approaches. Doctor Abell and I (1) could show this objectively by observing the vessels in windows made according to the Clark principle. These had been placed in the ears and mesenteries of rabbits and the mesenteries of cats. The mechanism by which this vasoconstriction occurs is not clear, but the fact that it is so persistent suggests that, in part, it is due to continuous liberation of a vasoconstrictor substance.

For this reason it seemed desirable to ascertain whether such a substance could be demonstrated in the blood of animals in shock induced by a variety of means. The perfused rabbit's ear method (2) was employed for demonstrating the presence of vasoconstrictor substance in dog's blood. Shock was induced by *1*, tourniquets applied to the limbs; *2*, severe hemorrhage; *3*, stripping of the intestines, and *4*, burns.

METHODS. Shock was produced in dogs under pentobarbital anesthesia (35 mgm./kgm. body weight) first by tourniquets placed around the hind limbs of dogs. The tourniquets were sufficiently tight to reduce arterial pressure distal to the obstruction to 30 mm. Hg or less and all return flow was not cut off. They were left in place for $3\frac{1}{2}$ to $4\frac{1}{2}$ hours. Blood samples were taken into syringes moistened with sodium citrate solution and coagulation was prevented by addition of 0.3 cc. of 10 per cent sodium citrate to every 5 cc. of blood. Shock was also produced by bleeding dogs up to as much as 7 per cent of their body weight and keeping the arterial pressure at 30 mm. Hg for 40 minutes. The details of this method will be described by Kohlstaedt and Page (3).

Stripping and exposure of the intestine has been used to produce shock. Dr. Robert Taylor, who is studying this method, supplied the blood samples. Shock was also produced by burning the extremities of anesthetized dogs by immersion in boiling water for from 20 seconds to 3 minutes.

Samples of citrated plasma were perfused through the isolated rabbit's ears using either calcium-free Ringer's solution or citrated normal dog's plasma as perfusing medium. Perfusion pressure may be kept very low (10/0 mm. Hg) when citrated plasma is used instead of defibrinated blood. Vasoconstriction in the ear was measured by the length of time required for the drop rate to be restored to its initial value as measured before the test injection, and by the

percentage reduction of drop rate after the injection as compared with the rate before.

All of the plasma samples (0.1 to 0.2 cc.) to be tested were first injected into the ear perfused with calcium-free Ringer's solution. After the intensity of the vasoconstriction had been thus ascertained, citrated plasma from normotensive dogs was substituted for the calcium-free Ringer's solution and the plasma samples again tested. This was done because at least two kinds of vasoconstrictor substances appear in blood. One occurs after blood has clotted (4) and causes vasoconstriction in the ear when either Ringer's solution or plasma perfuses it. The other, which occurs in blood of hypertensive patients and dogs, causes significant vasoconstriction only when plasma or blood are the perfusing media (5).

RESULTS. 1. *Tourniquet shock.* Blood samples from 32 dogs in shock have been studied. They were taken within 20 minutes after the tourniquets were in place and at intervals for several hours.

Plasma from these animals caused vasoconstriction within $\frac{1}{2}$ hour after applying the tourniquets both with calcium-free Ringer's solution and plasma as perfusing media (table 1). The amount of vasoconstrictor appeared to increase during the first hour but from then until death of the animal—usually from one to five hours after release of the tourniquets—no further increase occurred. No relationship was found to the level of arterial pressure.

Seven additional experiments were performed in which both kidneys were removed the day before tourniquet shock was induced (example in table 1). The vasoconstrictor appeared in the blood just as it had in intact animals. Three experiments were then done in which the left renal pedicle was denervated and the right kidney removed three days before the experiment. Again application of tourniquets and production of shock caused the appearance of the vasoconstrictor. Even section and partial destruction of the spinal cord from the second thoracic segment caudad failed to prevent its appearance when shock occurred.

2. *Hemorrhage.* From 2 to 7 per cent of the body weight of blood of the dog was removed rapidly. Arterial pressure usually fell to 30 to 40 mm. Hg but tended to rise if more blood was not removed. Within 5 minutes after such a severe hemorrhage, vasoconstrictor was found in the blood (table 2). It persisted throughout the period of hypotension. We have not determined how long it remains after blood pressure was restored by transfusion. Eighteen animals were used for these experiments.

In 5 additional dogs bilateral nephrectomy was performed varying from one day to one hour before bleeding. Vasoconstrictor appeared in the blood as in intact dogs.

Six experiments were also done in which both adrenal glands were removed from 1 day to 3 hours before the experiment. Due to susceptibility of these animals to vasomotor collapse it was necessary to bleed them more cautiously than the normal dogs. The operation did not interfere with the appearance of the vasoconstrictor substance in the blood.

TABLE 1

Formation of vasoconstrictor as a result of shock produced by constricting the blood supply to limbs of dogs

DOG NO.	B.P.	HEMA-TOCRIT	TIME	PROCEDURES	PLASMA PERFUSED		RINGER'S SO-LUTION PERFUSED	
					Re-duction of flow	Per cent re-duction of drop rate	Re-duction of flow	Per cent re-duction of drop rate
	mm. Hg				*minutes*		*minutes*	
16-22	234	49	8:30	Control	½	20	0	0
			8:40	Cords on legs				
	142		12:15	Released				
	122	75	3:10					
	130	73	4:00		7	59	6	44
	110	73	6:00		6	31		
	86	70	7:10	Died 6 hrs. later	19	49	7	70
16-24	165	37	4:30		0	0	1½	22
			4:45	Cords on legs				
	124	56	7:00		9	45		
	104	56	7:55		12	59		
			8:00	Released				
	66	61	8:45		12	54	6	68
	56	62	9:06	Died 1 hr. later	7½	40		
16-12	190	50	8:00	Bilateral nephrectomy 1 day before	2½	54	0	0
			8:10	Cords on legs				
	130	54	10:00		9	60	3	45
			11:05	Released	9	70	3	48
	62	59	11:15		9+	75	4	50
	58	60	1:00					
			1:30	Died suddenly				
16-46	148	40	7:10	Left renal denervation and right nephrectomy 3 days before	1	23	1½	36
			7:15	Cords on legs				
	124	62	9:15		2½	38		
	134	62	10:50		2½	36		
			11:00	Released				
	108	67	1:20		3	30		
	92	67	4:15		3	27		
	64	65	6:05		5	30	4½	60
16-43	122	42	11:30		0	0		
	44		12:00	Animal pithed				
			12:15	Cords on legs				
	44	47	1:30		4½	56		
	46	47	3:05					
	34		3:15	Cords released				
	32	51	3:40		2½	46		
	22	51	5:10		2½	47		

TABLE 2

The effect of hemorrhage in normal and nephrectomized dogs on the formation of vasoconstrictor

ORIGIN OF BLOOD	MEAN ARTERIAL PRESSURE	PLASMA PERFUSED		RINGER'S SOLUTION PERFUSED	
		Reduction of flow	Per cent reduction of drop rate	Reduction of flow	Per cent reduction of drop rate
	mm. Hg	*minutes*		*minutes*	
Normal dog					
Control....................................	160	1½	25	½	10
2 min. after 7.6 cc./kgm. blood removed..	40	¼	14		
1½ hours later.............................	42	3¾	44	5¼	36
10 min. after 3 cc./kgm. blood removed...	30	2	33		
10 min. after 3 cc./kgm. blood removed...	34	5½	46	5¾	83
10 min. after 3 cc./kgm. blood removed...	32	6½	48		
Same sample..............................		5¾	38		
Next day:					
Control....................................	110	2	38	1	32
1½ hrs. after 3 cc./kgm. blood removed ...	42	5	50	3	63
2½ hrs. later..............................	46	2½	36		
¾ hr. after 3 cc./kgm. blood removed	24	5½	46		
1¾ hrs. after 3 cc./kgm. blood removed ...	20	11	60		
Normal dog					
Control....................................	136	0	0	¾	34
200 cc. blood removed and sample taken 45 minutes later..........................	56	5¼	43	6½	88
Normal dog					
Control....................................	128	½	20	1½	22
200 cc. blood removed; sample taken immediately..................................	78	¾	12		
3 hours later.............................	40	3¼	42	2¼	64
Nephrectomized dog (24 hours before)					
Control....................................	142	1¼	28	0	0
1 hr. after 4 cc./kgm. blood removed	80	2	30	2	30
1 hr. after 4 cc./kgm. blood removed	72	2	55		
½ hr. later................................	60	1½	27		
1½ hrs. later..............................	78	1¼	7		
½ hr. later................................	46	3½	48	3¼	38 '
¼ hr. later................................	38	2¼	50		
Nephrectomized dog (3 hours before)					
Control....................................	130	1	19	1¼	32
Bled 3 per cent of body weight—20 minutes after bleeding.......................	42	4	47	3½	44
42 min. after bleeding....................	24	6	70		
68 min. after bleeding....................	50	2¼	47		
90 min. after bleeding....................	100				
All blood returned					
105 min. after bleeding...................	84	0	0		

Apparently adrenalin is either not liberated in large amounts or the method is too insensitive to detect small changes in it when hemorrhage occurs. This observation was unexpected since we had thought adrenalectomy might reduce the amount of vasoconstrictor found after hemorrhage.

3. *Intestinal stripping.* The results with this method for producing shock were the same as with the tourniquet method. Before the arterial pressure fell significantly, the plasma developed vasoconstrictor properties which appeared to persist until death of the animal. Four experiments of this type were performed (table 3). Ultra-filtrates prepared from citrated plasma produced as much vasoconstriction as the original plasma from which it was prepared.

4. *Burns.* Within 5 minutes after severe burns of the extremities from boiling water, the citrated plasma of the dogs showed marked hemoglobinemia. Vaso-constrictor action of the plasma was evident when perfused with calcium-free Ringer's solution through a rabbit's ear. Twenty-four experiments were done (table 3).

Since so much hemolysis had occurred as the result of the burns, it seemed desirable to ascertain how much this might contribute to the vasoconstrictor action of the plasma. Washed, packed red blood cells were laked with distilled water, the stroma removed by centrifuging, the supernatant solution added to plasma until the color matched to the naked eye that of the plasma from burned dogs. Such plasma caused slightly more vasoconstriction than did the control normal plasma but nothing like enough to account for the vasoconstrictor occurring in the plasma of burned animals.

Plasma from burned animals was subjected to ultra-filtration. The protein-free filtrate in quantities of 0.2 cc. caused usually as much vasoconstriction as did the original plasma. The constriction was more prolonged but seldom as sharp as that caused by the plasma itself.

For some unexplained reason several ultra-filtrates from plasma of burned animals showed no vasoconstrictor action. This was also true of serum, hence we are of the opinion that some uncontrolled factor affected the results. Since the majority of the ultra-filtrates showed marked vasoconstriction we are inclined to view the results as indicating that the vasoconstrictor is ultra-filtrable. This is strengthened by the fact that dialysis of plasma from burned dogs and normal serum for 17 hours in cellophane against normal salt solution causes disappearance of the vasoconstrictor action of the plasma or serum left within the dialysing sac (3 expts.).

If the ultra-filtrate was subjected to heat by immersing the tube containing it for 5 minutes in boiling water, the vasoconstrictor action was reduced but not abolished. About the same reduction in potency occurred when plasma was treated similarly, and after boiling, the coagulum filtered off. The vasoconstrictor substance is evidently not highly thermolabile.

5. *Dissimilarity of the vasoconstrictors occurring in plasma from shocked and burned dogs from those in hypertensives and in serum.* It has been demonstrated (5) that plasma and blood of hypertensive patients and dogs contains a vasoconstrictor substance as shown by the fact that they cause vasoconstriction

when perfused with normal plasma or blood through rabbits' ears. When Ringer's solution was used, very little vasoconstriction resulted. This fact aids

TABLE 3

Effect of burns and intestinal stripping in dogs on occurrence of vasoconstrictor in plasma (0.2 cc.) and plasma ultra-filtrates (0.2 cc.)

DOG NO.	TIME	PROCEDURES	PERFUSION MEDIUM	VASOCONSTRICTION IN RABBIT'S EAR	
				Reduction in drop rate	Per cent reduction of drop rate
				minutes	
19-25	Control	Anesthetized	Ringer's*	¼	16
	5 min.	After burn	Ringer's	16	75
	1 hour	After burn	Ringer's	4¼	79
	Control		Plasma	2	17
	5 min.	After burn	Plasma	8¾	81
	1 hour	After burn	Plasma	11½	82
19-23	Control	Anesthetized	Ringer's	1¼	34
		Intestine stripped	Ringer's	6½	85
		Ultra-filtrate	Ringer's	8¾	52
		Boiled ultra-filtrate	Ringer's	½	34
19-34	Control	Anesthetized	Ringer's	1½	23
	30 min.	After burning	Ringer's	6½	55
		Plasma heated in boiling water for 5 minutes	Ringer's	2½	35
		Ultra-filtrate	Ringer's	4½	59
		Plasma heated in boiling water for 5 minutes	Ringer's	3½	47
19-43	Control	Anesthetized	Ringer's	1¼	30
	10 min.	After burn	Ringer's	7	98
	1 hour	After burn	Ringer's	7¾	93
		Boiled 5 minutes	Ringer's	3½	81
		Ultra-filtrate	Ringer's	15	56
		Ultra-filtrate in boiling water-bath, 5 minutes	Ringer's	4¼	48
		Ultra filtrate	Ringer's	5	38
	2nd Assay		Ringer's		
	Control	Anesthetized	Ringer's	1	22
	1 hour	After burn. Plasma ultra-filtrate	Ringer's	10½	100
		After burn. Plasma	Ringer's	8¾	81
		Ultra-filtrate in boiling water-bath 5 minutes	Ringer's	3	55

* Calcium free.

in distinguishing the vasoconstrictor action of hypertensive's plasma from plasma drawn from shocked and burned dogs. But normal *serum* alone also causes vasoconstriction in the rabbit's ear when calcium-free Ringer's solution

perfuses the ear just as does plasma from shocked and burned animals. Confusion with histamine might also occur because it causes vasoconstriction under the conditions of our experiment. It was, therefore, desirable to determine whether the same vasoconstrictor occurred in blood of shocked and burned animals as that formed when blood coagulates. Further, it was necessary to distinguish these from histamine.

It was found that repeated injections of one type or another of plasma or serum ultimately causes the ear vessels of an occasional preparation to become refractory to further stimulation to this especial plasma or serum. Let us take, for example, the continued perfusion and injection of dog's serum until vasoconstriction no longer results. If now plasma from a burned dog was injected the full vasoconstrictor response was elicited. This principle was used to ascertain the similarity or dissimilarity of the various vasoconstrictors described in this investigation.

The rapidity with which refractoriness developed in different ears varied greatly. It seemed usually much easier to develop it in an ear perfused with plasma when hypertensive plasma was used than when normal serum was employed and the ear perfused with calcium-free Ringer's solution. As many as 60 injections of serum often failed to produce complete refractoriness in some ears. In order to facilitate its development, the experiments were conducted by perfusing for 30 minutes to an hour some of the plasma or serum to which refractoriness was desired, along with calcium-free Ringer's solution or normal citrated plasma. The experiments illustrated in the tables are those in which refractoriness developed quickly.

Refractoriness elicited to dog's serum abolished the vasoconstrictor action of human serum as well (table 4). But plasma from burned dogs and dogs in shock from tourniquets or hemorrhage still caused sharp vasoconstriction. Histamine also was active. It therefore appears that the vasoconstrictor in plasma of burned, bled, or shocked animals is not identical with that in dog or human serum. The vasoconstrictors in dog and human serum appear from this test to be identical. Histamine differs from the latter in that it continued to cause vasoconstriction after the ear was refractory to serum.

The next step was to perfuse ears with plasma from burned dogs and establish refractoriness to it (table 4). Human serum now gave full response while plasma from bled dogs gave none. Histamine was still active. These experiments, of which there were two, show again that the vasoconstrictor in burned animals differs from that in serum and that histamine is unlike both.

To show that the vasoconstrictor in hypertensive's blood differs from that in both shocked dog's plasma and in serum, ears were perfused with hypertensive dog's plasma until refractoriness developed. Repeated injections of human hypertensive's plasma was used to hasten it and also to ascertain the rate of development. When the ear no longer responded to either hypertensive human or dog's plasma, burned and bled dog's plasma still caused vasoconstriction (table 5). The same ear was then perfused with normal dog's plasma plus burned dog's plasma. Refractoriness to burned dog's plasma developed but now hypertensive's plasma again caused vasoconstriction. Human serum also

TABLE 4

Development of refractoriness to dog's serum and burned dog's plasma and the relationship to the vasoconstrictor response to plasma of burned dogs in hemorrhagic shock and histamine

SUBSTANCE INJECTED	AMOUNT	DROP RATE PER MINUTE	REDUCTION OF DROP RATE	PER CENT REDUCTION OF FLOW
(1) Ear perfused with calcium-free Ringer's solution plus dog's serum (0.15 cc. serum/cc. calcium-free Ringer's solution). Perfusion pressure 44/24 mm. Hg				
	cc.		*minutes*	
Dog serum.................................	0.2	48	$3\frac{1}{2}$	42
Dog serum.................................	0.2	48	1	25
Dog serum.................................	0.2	48	1	31
Human serum..............................	0.2	40	$1\frac{1}{4}$	22
Human serum..............................	0.2	44	0	0
Burned dog plasma.........................	0.2	44	8	82
Human serum..............................	0.2	48	0	0
Human serum..............................	0.2	48	0	0
Burned dog plasma.........................	0.2	48	6	76
Human serum..............................	0.2	60	0	0
Histamine (0.000027 mgm./cc.).............	0.2	60	4+	100
Human serum..............................	0.2	60	$\frac{1}{2}$	33
Human serum..............................	0.2	64	$\frac{1}{2}$	6
Dog plasma from hemorrhagic shock..........	0.2	64	2	55
Human serum..............................	0.2	60	0	0
Histamine (0.000027 mgm./cc.).............	0.2	60	$3\frac{1}{4}$	72
(2) Second ear perfused with calcium-free Ringer's solution for 30 minutes. Perfusion pressure 40/24 mm. Hg				
Human serum..............................	0.2	44	$2\frac{1}{2}$	47
Burned dog plasma.........................	0.2	44	$4\frac{1}{2}$	56
Dog plasma from hemorrhagic shock..........	0.2	48	6	77
Histamine (0.000027 mgm./cc.).............	0.2	60	6	33
Human serum..............................	0.2	52	$2\frac{3}{4}$	51
(3) Ear perfused with plasma from burned dog (0.25 cc. plasma/cc. calcium-free Ringer's solution). Perfusion pressure 64/46 mm. Hg				
Burned dog plasma.........................	0.2	44	3	55
Burned dog plasma.........................	0.2	44	$2\frac{1}{2}$	59
Burned dog plasma.........................	0.2	40	$2\frac{1}{2}$	—
Burned dog plasma.........................	0.2	36	2	—
Burned dog plasma.........................	0.2	36	1	—*
Burned dog plasma.........................	0.2	36	1	27
Burned dog plasma.........................	0.2	36	1	22
Burned dog plasma.........................	0.2	44	0	0
Human serum..............................	0.2	36	3	46
Dog plasma from hemorrhagic shock..........	0.2	40	0	0
Histamine (0.000027 mgm./cc.).............	0.2	40	$4\frac{1}{4}$	59
Burned dog plasma.........................	0.2	36	$\frac{1}{4}$	11
Dog plasma from hemorrhagic shock..........	0.2	36	0	0
Human serum..............................	0.2	36	2	31

* Given in rapid succession

caused vasoconstriction. These results suggest that the vasoconstrictors in dog and human hypertensives are similar but differ from those in burned or bled dog's plasma.

TABLE 5

Development of refractoriness to plasma of hypertensive patients and the relationship to the vasoconstrictor response to plasma of burned dogs and dogs with hemorrhagic shock

SUBSTANCE INJECTED	AMOUNT	DROP RATE PER MINUTE	VASOCONSTRICTION IN RABBIT'S EAR	
			Reduction of drop rate	Per cent reduction of flow
(1) Ear perfused with calcium-free Ringer's solution for 15 minutes				
	cc.		*minutes*	
Burned dog's plasma................	0.2	60	3	57
(2) Ear perfused with hypertensive dog's plasma (diluted 25 per cent with calcium-free Ringer's solution). Perfusion pressure 40/20 mm. Hg				
Human hypertensive's plasma........	0.2	13	4	35
Human hypertensive's plasma........	0.2	17	1	24
Human hypertensive's plasma........	0.2	17	1	17
Human hypertensive's plasma........	0.2	15	2	50
Hypertensive dog's plasma (another dog)............................	0.2	13	$1\frac{3}{4}$	23
Hypertensive dog's plasma..........	0.2	15	$1\frac{3}{4}$	28
Hypertensive dog's plasma..........	0.2	15	$\frac{1}{4}$	12
Human hypertensive's plasma........	0.2	15	0	0
Burned dog's plasma................	0.2	15	6+	100
Hypertensive dog's plasma..........	0.2	13	0	0
Human hypertensive's plasma........	0.2	15	0	0
Burned dog's plasma................	0.2	17	7	72
Hypertensive dog's plasma..........	0.2	13	0	0
Plasma from dog in hemorrhagic shock	0.2	13	$3\frac{1}{2}$	45
(3) Ear perfused with normal dog's plasma (9 parts) plus burned dog's plasma (1 part) 2 hours after last injection. Perfusion pressure 48/24 mm. Hg				
Burned dog's plasma................	0.2	13	5	61
Burned dog's plasma................	0.2	11	5	33
Burned dog's plasma................	0.2	13	1	8
Hypertensive dog's plasma..........	0.2	11	5	39
Burned dog's plasma................	0.2	13	0	0
Hypertensive dog's plasma..........	0.2	13	4	25
Burned dog's plasma................	0.2	13	$\frac{1}{2}$	13
Human serum......................	0.2	13	$3\frac{1}{2}$	58

6. *Use of the intestinal strip method.* Because of some uncertainty in the method just described, it seemed desirable to obtain confirmatory evidence using another method. The isolated rabbit's intestinal ring preparation was selected. Rings from 2 to 3 cm. in length were cut from the small intestine and kept

in Ringer's solution in the ice box until ready for use. The temperature of the 30 cc. cups were thermostatically controlled to within 0.1°C. Glucose containing, but calcium-free Ringer's solution, was used as the bathing medium. When a stable rhythm was established the test substance was added in 1 cc. doses.

The addition of dog or human serum causes sharp contraction of the gut while citrated plasma causes none in the doses employed. Citrated plasma from blood of burned dogs or dogs subjected to tourniquet shock caused no contraction. The same plasma had been tested on the perfused rabbit's ear and shown to cause marked vasoconstriction. These experiments, of which 9 were done, show that the vasoconstrictor in serum differs from that in plasma from burned and shocked animals.

DISCUSSION. The purpose of this investigation was to learn more about the mechanism by which vasoconstriction was induced when animals were in secondary shock, whether from tissue injury, hemorrhage or burns. It is apparent that plasma from animals in, or approaching shock produced by several methods, acquires vasoconstrictor properties as measured in the isolated perfused rabbit's ear.

The chemical nature of the vasoconstrictor is not known. Since it causes vasoconstriction when the ear is perfused with Ringer's solution it is unlikely that it is identical with the vasoconstrictor which occurs in experimental or clinical renal hypertension. It is probably ultra-filtrable and hence it might be assumed to be a crystalloid of not excessively high molecular weight. It is also not markedly heat-labile.

These properties are not unlike those of the vasoconstrictor substance which results when normal blood coagulates (4–6). The serum formed causes much greater vasoconstriction in the rabbit's ear than does the plasma.[1] Furthermore, vasoconstriction occurs when Ringer's solution is the perfusing medium, thus differentiating it from the vasoconstrictor of hypertensives whose action is dependent upon the presence of plasma or blood. The vasoconstrictor of serum can also be separated by ultra-filtration (5), an observation which we can confirm.

It is possible that the vasoconstrictor which occurs in shock, and that occurring when blood coagulates, are similar. In view of the widespread tissue destruction which occurs in burns, after application of tourniquets and after stripping of the intestine, it would not be surprising that chemical changes associated with coagulation might be initiated.

Another possibility should be considered; namely, that the vasoconstrictor is histamine. It is recognized that histamine can cause vasoconstriction in the vessels of the ears of intact rabbits (7, 8, 9) and we have found it to act similarly in the perfused ears under the conditions of our experiments.

Neither of these hypotheses receives any support from the experiments in

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[1] In a previous paper results were published which suggested that plasma and serum were equal in vasoconstrictor properties. This was an unfortunate mistake because many results both before and after showed the contrary, as, indeed, did the results in the literature. Dr. Eugene Landis was good enough to call our attention to this error.

which refractoriness was used as a method to identify a particular vasoconstrictor. This is an interesting method but one which has not as yet been intensively studied to ascertain its mechanism. It appears to depend on the fatiguing of certain elements in the vascular musculature by repeated injection of, and perfusion with, a particular plasma or serum. When the vessels no longer respond by vasoconstriction, the plasma or serum with which it is to be compared is injected. When no response occurs, it is taken as evidence in favor of similarity to the fatigue inducing plasma or serum. Sharp vasoconstriction, on the contrary, suggests dissimilarity.

It is worth digressing to point out that the development of refractoriness occurs at unusually different rates. Some ears continue to respond by vasoconstriction while perfusing with the fatigue-producing serum or plasma and after the injection of 60 or more samples of serum, while in a rare one refractoriness is elicited after several injections. The latter experiments seem to be the ones suited to demonstrate the similarity or dissimilarity of vasoconstrictors.

The importance of the method is greatly weakened by the fact that only certain ears cease to respond to the repeated stimulus readily and we have been unable to determine why this is so. It is thus not an easily reproducible phenomenon and correspondingly cannot be given as much weight as we might like. Nevertheless, when refractoriness develops, the results are so clear they can hardly be doubted. These reservations must be kept clearly in mind in the interpretation of the results pertaining to the dissimilarity of the vasoconstrictors in serum and shock plasma.

Whether this method gives proof of identity seems uncertain. It is conceivable that the vasoconstrictor could be the same but the quality or quantity of another substance present in plasma could so modify the vasoconstrictor action as to give misleading conclusions. Several years ago we showed (10) that in the perfused ear, plasma or blood was necessary for angiotonin to exert its full vasoconstrictor action. We postulated the presence of an "angiotonin-activator" in blood. This substance might have a potent orienting influence on the vasoconstrictor. It seems more reasonable, however, to assume that vasoconstriction can be elicited by a variety of mechanisms one or more of which may be fatigued by repeated stimuli. The fatigue of one does not preclude vasoconstriction occurring as a result of the action of another. It is this type of mechanism with which we appear to be dealing in these experiments.

The results of application of the method leave little doubt that, plasma from traumatized, bled and burned dogs contain similar vasoconstrictors which differ from that formed when blood coagulates. Both of these differ from that present in the plasma of chronic hypertensives. Furthermore, histamine differs from all of them. The only question is whether this method is a valid one.

Some support is offered by the work on intestinal strips. These respond regularly by severe contraction to serum but plasma causes little or none. Histamine also causes contraction. But plasma from burned or shocked animals does not cause any more contraction than plasma of normal dogs. The same plasma which elicited sharp constriction in the rabbit's ear was inactive on intestine.

Neither the kidneys nor the adrenal glands seem necessary for the formation of the vasoconstrictor for it occurs in their absence when shock is produced by hemorrhage and tourniquets. Nephrectomy, renal denervation, and low spinal cord destruction do not prevent its appearance in tourniquet shock. It seems not unreasonable to suppose that the occurrence of a vasoconstrictor arising in shock is associated with anoxia of the tissues, since this is at least one of the factors common to shock produced by these different methods.

It is of great interest that Sapirstein, Ogden and Southard (11, 12) have presented evidence that hemorrhage causes the renin content of the blood to increase; possibly as a "homeostatic" effort on the part of the body to restore blood pressure to normal levels. Hamilton and Collins (13) have shown that hemorrhage imparts *pressor* activity to blood as tested on nephrectomized dogs. This occurs in normal and adrenalectomized animals as well as in those with the kidneys denervated. The type of pressor response was suggestive of the presence of both renin and angiotonin. The kidneys were the source of these substances because *1*, renal vein blood usually gave greater responses than the corresponding arterial blood, and *2*, pressor reactions were obtained from the blood of adrenalectomized dogs, but not from dogs with both kidneys and adrenal glands removed. Huidobro and Braun-Menendez (14) also found renin liberated by hemorrhage in intact anesthetized dogs. Renin could be detected in the systemic blood of these dogs but not in nephrectomized animals after hemorrhage.

These results, in conjunction with our own, suggest that at least two substances are involved in hemorrhagic shock, one from the kidneys having pressor qualities and the other from elsewhere in the body having vasoconstrictor properties.

SUMMARY. 1. Shock has been produced in dogs by means of *a*, tourniquets placed around the hind extremities; *b*, stripping and exposure of the intestines; *c*, hemorrhage; *d*, burns.

2. Vasoconstrictor properties develop in the blood of such animals which can be demonstrated by perfusing samples of citrated plasma through isolated perfused rabbits' ears. Vasoconstriction results regardless of whether the ears are being perfused with calcium-free Ringer's solution or by citrated plasma from normal dogs.

3. Renal denervation, nephrectomy, adrenalectomy and low spinal destruction do not prevent the appearance of vasoconstrictor after tourniquet shock. Nor does nephrectomy and adrenalectomy prevent it after hemorrhage.

4. Refractoriness to the vasoconstrictor action of sera and plasma may be produced by perfusion with, and repeated injections of them. Once established, the vessels of the rabbit's ear still respond to plasma of different origin. This method has been used to ascertain similarity or dissimilarity among the various plasma and sera containing vasoconstrictors.

5. Refractoriness to serum does not abolish the vasoconstrictor action of plasma from burned, bled or shocked dogs, or to histamine. Refractoriness to plasma from burned dogs does not abolish the vasoconstrictor action of serum or histamine but does do so to plasma from bled or hypertensive dogs. Re-

fractoriness to hypertensive's plasma does not abolish the vasoconstrictor action of burned or bled dog's plasma.

6. Rabbits' isolated intestinal rings contract powerfully when serum is added to calcium-free Ringer's solution bathing them. Normal citrated plasma and plasma from burned or shocked dogs causes no contraction in dilutions of 1 to 30.

CONCLUSIONS

Shock, whether elicited by tourniquets placed around the extremities, stripping and exposing the intestines, hemorrhage and burns, is associated with the appearance in the plasma of a substance which causes vasoconstriction in rabbits' ears perfused with either calcium-free Ringer's solution or plasma. It does not originate in the kidneys, adrenal glands, nor does destruction of the spinal cord or renal denervation prevent its appearance. Evidence gathered from application of a method depending on "fatiguing" the vascular musculature suggests, if the validity of the method is acceptable, that the vasoconstrictor action of plasma from burned, bled and shocked dogs is caused by identical or very similar substances. Furthermore, it differs from the vasoconstrictors present in hypertensive's (human and canine) plasma and in serum (human and canine). None of these vasoconstrictors seem to be histamine. This belief is confirmed by experiments on isolated intestine which show that serum causes marked contraction while plasma from burned or shocked dogs causes none.

REFERENCES

(1) PAGE, I. H. AND R. G. ABELL.   J. Exper. Med. 77: 215, 1943.
(2) PAGE, I. H.   Am. Heart J. 23: 336, 1942.
(3) KOHLSTAEDT, K. G. AND I. H. PAGE.   Arch. Surg. (in press).
(4) O'CONNOR, J. M.   Arch. exper. Path und Pharmakol. 67: 195, 1912.
(5) PAGE, I. H.   J. Exper. Med. 72: 301, 1940.
(6) JANEWAY, I. C., H. B. RICHARDSON AND E. A. PARK.   Arch. Int. Med. 21: 565, 1918.
(7) REID, G. AND M. BECK.   Med. J. Australia 1: 245, 1942.
(8) FELDBERG, W.   J. Physiol. 63: 211, 1927.
(9) WILSON, H. C.   J. Pharmacol. and Exper. Therap. 56: 97, 1936.
(10) PAGE, I. H. AND O. M. HELMER.   J. Exper. Med. 71: 495, 1940.
(11) SAPIRSTEIN, L. A., E. OGDEN AND F. D. SOUTHARD.   Proc. Soc. Exper. Biol. Med. 48: 505, 1941.
(12) SAPIRSTEIN, L. A., F. D. SOUTHARD AND E. OGDEN.   Proc. Soc. Exper. Biol. Med. 50: 320, 1942.
(13) HAMILTON, A. S. AND D. A. COLLINS.   This Journal 136: 275, 1942.
(14) HUIDOBRO, F. AND E. BRAUN-MENENDEZ.   This Journal 137: 47, 1942.

EFFECT OF COBALT ON WORK PERFORMANCE UNDER CONDITIONS OF ANOXIA[1]

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In an earlier report a method for measuring the work performance of rats under conditions of anoxia was described in detail (1). This method consisted, essentially, of exercising rats to the endpoint of exhaustion in circular, motor driven cages in an atmosphere of 6.8 per cent oxygen (52 mm. Hg partial pressure).

The development of polycythemia in animals and humans living at high altitude has long been regarded as an important factor in successful adaptation to such an environment. Recent studies indicated that administration of cobaltous ion in small doses induced polycythemia (2, 3, 4, 5, 6). It seemed worthwhile, therefore, to determine if the polycythemia induced by cobalt administration facilitated work performance under conditions of reduced oxygen tension.

METHODS. In these experiments, male rats of the McCollum strain were maintained on a constant synthetic diet which contained adequate quantities of minerals and vitamins. The method of conducting work performance tests under conditions of reduced oxygen tension has been described (1). After a suitable training period ten animals were selected on the basis of reproducibility of performance. Cobalt ($CoCl_2 \cdot 6H_2O$) was given in the drinking water to five of these animals. The dose of cobalt was so adjusted that each cubic centimeter of drinking water contained 100 gamma of cobaltous ions. Cobalt administration was continued by this method for fourteen weeks, except for a period from the second to fourth weeks during which 0.6 mgm. of cobaltous ions in 0.1 cc. of normal saline was injected subcutaneously daily. During this period, tap water was substituted for the cobalt solution. The remaining five animals drank tap water during the entire experiment and were used as controls.

Measurements of work performance, in an atmosphere consisting of 6.8 per cent oxygen (52 mm. Hg partial pressure) and 93.2 per cent nitrogen, were made at appropriate intervals during the period of cobalt administration and for six

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weeks after cobalt was discontinued. In these experiments it was found advantageous to observe the animals during work performance tests for five minutes after they appeared exhausted, rather than for two minutes as suggested in an earlier report (1). The five trained animals which were used as controls for the work experiments were always tested at the same time as the cobalt treated animals.

Two additional groups of nine animals each were used as controls. One group received cobalt without work performance tests. Cobalt treatment in this group differed slightly from that in the work performance test group in that oral administration was used exclusively except during the first week. The remaining group received neither cobalt nor work performance tests.

Hemoglobin was determined as acid hematin by means of a photoelectric colorimeter. Blood specimens for these determinations were obtained from the cut surface of the tip of the tail.

Complete autopsies were performed on all animals. The two groups of animals which underwent work performance tests were sacrificed six weeks follow-

TABLE 1

Effect of cobalt treatment on hemoglobin values of rats

GROUP	DURATION OF COBALT TREATMENT				
	2 weeks	6 weeks	8 weeks	11 weeks	14 weeks
Work performance test (anoxia) plus cobalt (5 animals)...................................	10.9	20.4	21.3	20.9	21.6
Work performance test (anoxia) alone (5 animals)..		16.3	14.8	15.8	15.5
Cobalt alone (9 animals)........................	16.7	16.8	19.1	20.5	
Controls (9 animals)............................	14.9	15.4	15.6	15.8	

ing withdrawal of cobalt. The animals which did not participate in work performance studies were sacrificed immediately following eleven weeks of cobalt, at a time when their hemoglobin response was at its maximum.

OBSERVATIONS. *Effect of cobalt on hemoglobin values.* As indicated by earlier workers, administration of cobalt to normal rats was followed by a striking polycythemia (table 1). After five to six weeks of cobalt the hemoglobin of the five treated animals (work performance test group) rose from an average initial value of 15.6 grams per 100 cc. of blood to 20.4 grams per 100 cc. of blood. During this period there was no significant change in the hemoglobin value of control rats (work performance test group). In the group of nine animals treated with cobalt but not given work performance tests, hemoglobin values rose from an average level of 16.7 grams to 19.1 grams after eight weeks of cobalt (table 1). It is probable that the longer period of cobalt administered by the parenteral route in the work performance test group was responsible for the more rapid development of polycythemia in this group of animals. Both groups of animals, however, ultimately attained the same hemoglobin levels at the end of eleven weeks of treatment.

Polycythemia persisted as long as cobalt was continued in all of the animals except one. This animal's hemoglobin value fell to 13.8 grams during the eleventh week of cobalt treatment after having attained the high value of 19.0 grams during the eighth week.

Following cessation of cobalt, the polycythemia gradually subsided. A group of five rats with average hemoglobin values of 21.6 grams per 100 cc. at the end of fourteen weeks of cobalt administration showed average hemoglobin values of 18.1 grams three weeks after cobalt was discontinued (table 2).

Effect of cobalt on body weight. During fourteen weeks of cobalt administration the five work performance test animals gained an average of 82 grams, whereas the five work performance test animals which did not receive cobalt gained an average of 117 grams. Following cessation of cobalt treatment, the work performance test group displayed an accelerated rate of weight gain which enabled them within a period of six weeks to equal the controls in weight. No alteration in the rate of

TABLE 2

Changes in hemoglobin values following withdrawal of cobalt

ANIMAL	AT END OF 14 WEEKS OF COBALT TREATMENT	FOLLOWING WITHDRAWAL OF COBALT TREATMENT		
		1 week	2 weeks	3 weeks
1	23.0	22.2	22.0	20.4
2	23.5	21.6	18.8	18.0
3	22.0	20.2	15.0	18.6
4	20.6	20.0	17.5	18.6
5	19.0	19.0	18.9	14.9
Mean	21.6	20.6	18.4	18.1

growth was observed between the cobalt treated controls (not given work performance test) and the totally untreated group. These data indicate that with this dose of cobalt, retardation in growth did not occur unless an added stress (work performance test in anoxia) was present. Treatment with larger doses of cobalt alone or exposure for longer periods to anoxia alone retarded growth.

Effect of cobalt on work performance under conditions of anoxia. On two successive tests of base-line performance five animals selected for cobalt treatment averaged 11.3 and 12.2 minutes respectively, and the control animals averaged 9.2 and 11.0 minutes respectively (chart 1).

During the first four weeks of cobalt treatment the work performance of both cobalt treated and control animals decreased (chart 1). Coincident with the appearance of increased hemoglobin values (fifth to sixth week) work performance of the cobalt treated animals improved, reaching a maximum of 17.3 minutes during the seventh week of cobalt. This improvement in work performance of the group resulted from an increase in work performance of three of the five animals tested. Continuation of cobalt administration thereafter failed to

induce further improvement. In contrast, the control animals failed to exceed their base-line performances at any time during this fourteen-week period.

Shortly *after cessation* of cobalt treatment there was a striking increase in the work performance of *all* of the polycythemic rats. Measurements made during the first week after withdrawal of cobalt showed an average performance of 23.6 minutes, an improvement of 100 per cent over the mean base-line values. During this period the control animals showed no significant change in work performance.

The increased ability of the cobalt treated animals to perform work under conditions of anoxia did not appear to depend on cardiac hypertrophy since the heart weight in the two groups of animals was almost identical, i.e., 300 mgm./100 grams of animal in the cobalt treated group; 297 mgm./100 grams of animal in the
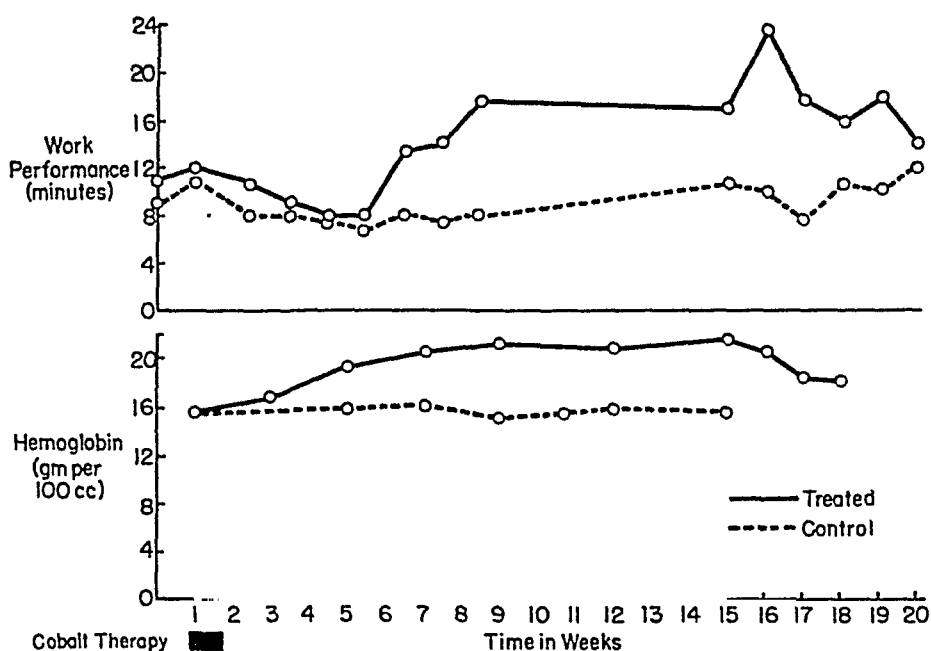
Chart 1. Effect of cobalt therapy on work performance and hemoglobin values of rats.

untreated control group. No significant difference in thymus weight was observed in the two groups of animals, i.e., 66 mgm./100 grams of animal in the cobalt treated group; 62.7 mgm./100 grams of animal in the control. There did appear to be a slight reduction in the weight of the adrenal glands in the cobalt treated animals, i.e., adrenal weight was 9.9 mgm./100 grams of animal in the cobalt treated group; 11.5 mgm./100 grams of animal in the control group. The cobalt treated group of animals weighed 344 grams (average), the control 351 grams (average).

Effect of cobalt on the hematopoietic system. The nine animals receiving cobalt but no work performance tests were autopsied immediately following eleven weeks of treatment. The only prominent gross finding was generalized vascular congestion of all tissues. There was slight enlargement of the spleen. The outstanding microscopic finding in these animals was a widespread increase in

erythropoietic activity. This was marked in the spleen (fig. 2), moderate in the bone marrow (fig. 4) and slight in the liver. The spleen showed depletion of the sheaths of lymphocytes that border the Malpighian corpuscles. In addition to these changes there was diffuse congestion of the lungs and kidneys. No evidences of injury, toxic or otherwise, were observed in the heart, liver and kidneys. In the control group of animals autopsied at the same time no evidence of abnormal or increased erythropoiesis was encountered (fig. 1 (spleen) and fig. 3 (bone marrow)).

All animals of the work performance groups were autopsied six weeks after the withdrawal of cobalt from the cobalt treated animals. Those which had received cobalt manifested hemosiderosis of the spleen, bone marrow and thymus, which may be considered to be indicative of an increase in blood destruction in these animals. No evidence of abnormal or increased erythropoesis was present in any organs of these animals. The spleens were almost twice as large as those encountered in any other group of rats in this study (fig. 5). Microscopically, this enlargement seemed to be due solely to vascular congestion. The Malpighian corpuscles were normal. Animals which had been tested for work performance under conditions of anoxia but had not undergone cobalt treatment showed no pathological differences from the control group not subjected to work performance tests.

DISCUSSION. In these experiments the effect of cobalt in increasing the hemoglobin of normal animals was confirmed. The increased erythropoietic activity appeared to be most marked in the spleen, moderate in the bone marrow and slight in the liver. This is at variance with other reports (6, 7) which state that the spleen plays no essential rôle in this phenomenon. It is quite possible that this apparent discrepancy in observations may be explained on the basis of differences in dosage and duration of treatment. It is of interest to note that in one animal, the hematopoietic system was apparently depressed by cobalt after an initial stimulation. This inhibition of hematopoiesis resembled that recently noted in anemic dogs to whom cobalt had been administered (8).

Cobalt treated rats performing work under conditions of anoxia failed to gain weight as rapidly as control animals. Cobalt treatment alone did not influence the rate of growth of rats when the animals were not forced to perform work in an atmosphere of reduced oxygen tension. It is possible, therefore, that a latent toxicity of the cobalt ion was unmasked when animals treated with cobalt were subjected to repeated stress and strain.

An increase in the work performance in an atmosphere of reduced oxygen tension occurred in three of five cobalt treated rats, after polycythemia developed. When cobalt was withdrawn for a period too short to permit a significant fall in hemoglobin (1 wk.) a marked improvement in work performance of all five of the polycythemic animals occurred. It appeared probable, therefore, that cobalt influenced work performance favorably by inducing polycythemia, and unfavorably by some inhibiting effect. The improved performance achieved by animals under cobalt appears to be the resultant of these antagonistic actions. The striking increase in size of the spleen in rats subjected both to work per-
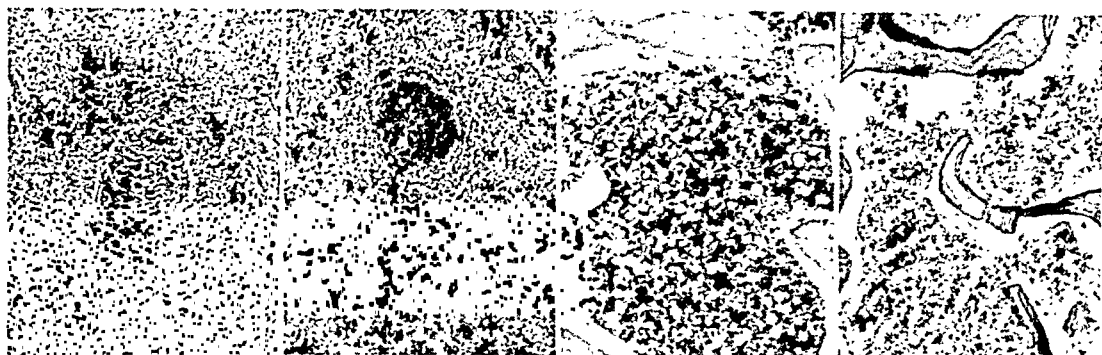
Fig. 1   Fig. 2   Fig. 3   Fig. 4

Fig. 1. Spleen of normal control rat.

Fig. 2. Spleen of rat subjected to cobalt treatment and to work performance tests, showing many islands of erythropoiesis in the red pulp, and lymphoid depletion, particularly around the follicles.

Fig. 3. Femoral marrow of normal control rat.

Fig. 4. Femoral marrow of rat subjected to cobalt treatment and to work performance tests, showing disappearance of fat and increase in foci of erythropoiesis.

All figures $\times$ 47, hematoxylin-eosin stain.

Fig. 5. Spleens from two rats representative of each group. Weights are given as recorded after fixation in formalin.

Top pair: cobalt and work performance tests; 1.01 grams, 1.28 grams.

Upper mid pair: cobalt only; 0.81 gram; 0.72 gram.

Lower mid pair: work performance tests only; 0.51 gram, 0.59 gram.

Bottom pair: normal control; 0.65 gram, 0.57 gram.

formance tests and to cobalt treatment is not open to simple interpretation, as the experiment included no rats allowed to recover from cobalt treatment without work tests.

CONCLUSIONS

1. Cobaltous ion in small doses induced erythropoietic activity in the spleen and liver and increased erythropoiesis in the bone marrow of normal rats. Significant increases in hemoglobin value were observed.

2. Animals so treated were observed to have an increased work performance under conditions of anoxia.

3. Although cobaltous ion in the dosage employed did not retard the growth of normal rats, some evidence of an untoward effect associated with its administration was indicated by the steady improvement in work performance which was noted immediately after withdrawal of cobalt and prior to the fall in hemoglobin values.

4. Enlargement of the spleen, apparently caused by vascular congestion, appeared in rats which were subjected to work performance tests and cobalt treatment and which were then allowed to continue six weeks after withdrawal of cobalt.

REFERENCES

(1) DORRANCE, S. S., G. W. THORN, F. H. TYLER AND B. S. KATZIN. Endocrinology **31:** 209, 1942.
(2) WALTNER, K. AND K. WALTNER. Klin. Wchnschr. **8:** 313, 1929.
(3) ORTEN, J. M., F. A. UNDERHILL, E. R. MUGRAGE AND R. C. LEWIS. Proc. Soc. Exper. Biol. and Med. **29:** 174, 1931.
(4) SUTTER, J. Compt. rend Soc. de Biol. **116:** 994, 1934.
(5) KLEINBERG, W. This Journal **108:** 545, 1934.
(6) SHILS, M. E. AND E. V. McCOLLUM. J. A. M. A. **120:** 609, 1942.
(7) ORTEN, J. M. This Journal **114:** 414, 1936.
(8) ROBSCHEIT-ROBBINS, F. S. AND G. H. WHIPPLE. J. Exper. Med. **75:** 481, 1942.

THE INFLUENCE OF PREGNANCY, HYPERVITAMINOSIS-D AND PARTIAL NEPHRECTOMY ON THE VOLUME OF THE PARATHYROID GLANDS IN RATS

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A review of the literature indicates that three factors appear to be concerned with an increase in size and function of the parathyroid glands. That the glands undergo hyperplasia in chronic renal disease has been shown by Castleman and Mallory (1) on the basis of clinical material. A significant increase in glandular volume was demonstrated experimentally following partial nephrectomy in rats by Pappenheimer (2). Others have found that hyperparathyroidism, the functional counterpart of hyperplasia, exists in human beings with renal insufficiency as well as in dogs which have been subjected to experimental partial nephrectomy (3).

The relationship between pregnancy and the parathyroid glands is more obscure. It has been stated that hyperparathyroidism is three times more common in women and that it occurs with greatest frequency during the childbearing age (4). Evidence for a physiologic increase in parathyroid activity during normal gestation is clinical rather than experimental. Several workers appear to have demonstrated abnormally large amounts of parathyroid extract in the blood of pregnant women during the last trimester (5). Hyperplasia and increased vascularity of the glands during pregnancy have also been observed (6).

A third factor which is said to influence the size and function of the parathyroid glands is vitamin D. A number of investigators (7) have shown that a deficiency of this vitamin in chickens leads to a hyperplasia and it has been postulated that this effect represents a compensatory mechanism. It is thought that stimulation of the parathyroid apparatus constitutes an effort on the part of the organism to elevate the Ca-P levels of the blood, lowered by vitamin D deficiency. Evidence of increased function in vitamin-D deficient children is provided by Hamilton and Schwartz (8).

There are others, however, who hold to the theory that vitamin D, rather than the lack of it, stimulates the parathyroid glands. It is pointed out by these workers (9) that the administration of this vitamin helps to alleviate the ill effects of parathyroidectomy. Grant and Gates (10) found that the exposure of rabbits to ultraviolet light consistently caused a hyperplasia of the parathyroid glands, amounting in some instances to more than half the normal weight.

EXPERIMENT. In order to determine the relative effects of renal injury, vitamin D and pregnancy upon the size of the parathyroid glands, 6 groups of rats were used.

The group of normal controls consisted of 9 littermates of the rats which

formed the second, or partially nephrectomized, group. The method of reducing substantially the amount of functioning renal tissue in the latter group was the same as described in a previous experiment (11). The left kidney was removed under ether anesthesia when the animal weighed from 60 to 80 grams. In a second operation, following the first by approximately one week, the upper half of the right kidney was ligated.

A third group of rats was fed an average of 100,000 I.U. of irradiated ergosterol over a period of 27 days prior to autopsy. This was in addition to their balanced basal ration. The same excessive amount of vitamin D was given to paired littermates of this group, the members of which were previously subjected to partial nephrectomy. This fourth group was set up to evaluate the effect of summation of vitamin D and nephrectomy in the same animal.

A fifth group of 10 female rats were made pregnant and autopsied within the last 2 or 3 days of gestation. Ten paired littermates of group 5 were similarly

TABLE 1

Volume, of parathyroids, summary of statistical data

GROUPS	NUMBER IN GROUP	MEAN VOLUME $\times 10^5 \mu$	STANDARD DEVIATION OF MEAN $\times 10^5 \mu$
Untreated control......................	9	114	10.6
Nephrectomy............................	9	302	48.6
Vitamin D.............................	7	151	18
Vitamin D with nephrectomy............	7	390	92.8
Pregnancy..............................	10	161	12.8
Pregnancy with nephrectomy............	10	259	25.8

The usual statistical formulae were used throughout; in obtaining standard deviations, the correction $(n - 2)$ was used for groups of ten members, and $(n - 3)$ for groups with less than ten members.

autopsied during the last days of pregnancy, the onset of which followed partial nephrectomy. Again, this dual procedure was employed to determine the combined effects of the 2 factors, pregnancy and renal injury.

The rats of all 6 groups were killed by exsanguination.[1] The parathyroids with attached thyroid tissue were removed, fixed in Bouin's solution, sectioned serially at 6 μ and stained with hematoxylin and eosin.

The volume of the parathyroids was determined by the method described by Meredith (12), whereby interval sections from the glands were projected at a uniform magnification. The areas of projection were determined by means of planimeter and curves drawn for each organ using the serial position of the section as measured in micra as the abscissa and the area of projection of that section as the ordinate. A smooth curve was drawn connecting these points and the area under the curve, measured by means of planimeter. From this figure, an index of the total volume of parathyroid tissue was easily derived.

RESULTS. A summary of the statistical data (table 1) reveals the largest standard deviations within those groups of rats which were subjected either to

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[1] Variations in weight and age at autopsy were kept within a very narrow range.

nephrectomy alone, or to nephrectomy in conjunction with some other treatment. This increased intra-group variation is probably related to an accidental difference in the quantitative reduction of functional renal substance. Pappenheimer and Wilens (13) in their studies of 21 cases of chronic renal disease found an increase in the weight of the parathyroids roughly proportional to the severity and extent of the renal lesions and to the degree of clinical insufficiency. In this experiment, ligation of the second kidney was designed to produce comparable increased reductions of renal substance. That this procedure could not obtain identical degrees of damage in a series of animals was expected.

In lines a, b and c of table 2 a comparison is made between groups which differ only in respect to nephrectomy. In each instance the volume of the parathyroids is greater in the nephrectomized group. This excess amounts to 165 per cent in the series not otherwise treated, 158 per cent in the vitamin D treated

TABLE 2

Comparisons between groups

COMPARISON	DIFFERENCE $\times 10^5 \mu$	S.D OF DIFFERENCE $\times 10^5 \mu$	CRITICAL RATIO
a. Nephrectomy minus control..........	188	49.7	3.78
b. Vit. D & nephr. minus vit. D........	239	94.5	2.53
c. Preg. & nephr. minus preg..........	98	28.8	3.40
d. Preg. minus control................	47	16.6	2.83
e. Vit. D minus control...............	37	20.9	1.77
f. Vit. D & nephr. minus nephr.........	88	104.6	0.84
g. Nephr. minus preg. & nephr.........	43	55	0.78

rats, and 61 per cent in the pregnant animals. These differences have a critical ratio over three in the untreated and pregnant series, and are therefore statistically highly significant. The critical ratio of 2.53 in the vitamin D treated series indicates that the difference is fairly significant.

The difference between the untreated control group, and the other groups subjected to nephrectomy, pregnancy or hypervitaminosis-D above is brought out in lines a, d and e. Line a has already been discussed. Differences between animals which were not littermates but which were of comparable age and weight at autopsy are presented in lines d and e.

The volume of the parathyroids in the pregnant group exceeds that in the control group by 40.5 per cent. This difference is fairly significant. The glandular volume of the hypervitaminotic group exceeds that of the control group by 31.5 per cent but the difference is not very significant since the critical ratio falls below 2.0.

Tabulation of the difference among the nephrectomized groups in lines f and g reveals that the nephrectomized group lies midway between the pregnant-nephrectomized group and the vitamin D-nephrectomized group, but does not differ from either to a statistically reliable degree. The smallest volume is

found in the pregnant-nephrectomized rats which gives a value 14.2 per cent less than that for the nephrectomized animals. The nephrectomized group is exceeded by 29.3 per cent by the vitamin D-nephrectomized group.

As it is reasonable to assume that glandular and body weight are correlated, the tendency for nephrectomized rats to weigh 20 to 30 grams less than their non-nephrectomized littermates might serve to decrease slightly the volume of the parathyroids in the former. This would, if anything, further enhance the significance of the tabulated results.

SUMMARY AND CONCLUSIONS

The significance of the three factors to which hyperplasia of the parathyroid glands is ascribed in the literature was tested experimentally in 52 rats, divided into 6 groups. The groups were subjected to no treatment, partial nephrectomy, hypervitaminosis-D, pregnancy, nephrectomy and hypervitaminosis-D, and nephrectomy and pregnancy, respectively.

Partial nephrectomy was associated with a statistically reliable increase in parathyroid volume in rats not otherwise treated as well as in groups which were also made hypervitaminotic or pregnant.

Pregnancy alone was associated with a fairly significant increase in parathyroid volume.

Hypervitaminosis-D alone in rats was associated with a statistically unreliable increase in parathyroid volume.

Within the nephrectomized groups, there was no significant difference between the group not otherwise treated and either that given vitamin D or made pregnant.

A summation effect of two factors, renal damage and pregnancy, each capable of producing hyperplasia of the parathyroids, was not apparent.

REFERENCES

(1) CASTLEMAN, B. AND T. B. MALLORY. Am. J. Path. **13**: 553, 1937.
(2) PAPPENHEIMER, A. M. J. Exper. Med. **64**: 965, 1936.
(3) HIGHMAN, W. J., JR. AND B. HAMILTON. J. Clin. Investigation **16**: 103, 1937. Arch. Path. **26**: 1029, 1938.
(4) WILDER, R. M. AND L. P. HOWELL. J. A. M. A. **106**: 427, 1936.
(5) HAMILTON, B., L. DeSEF, W. J. HIGHMAN, JR. AND C. SCHWARTZ. J. Clin. Investigation **15**: 323, 1936.
HOFFMAN, F. Arch. f. Gynäk. **153**: 181, 1933.
(6) SEITZ, L. Arch. f. Gynäk. **89**: 53, 1909.
(7) NONIDEZ, J. F. AND H. D. GOODALE. Am. J. Anat. **38**: 319, 1927.
HIGGINS, G. M. AND C. SHEARD. This Journal **85**: 299, 1928.
HIGGINS, G. M., C. SHEARD AND R. M. WILDER. Anat. Rec. **58**: 205, 1934.
(8) HAMILTON, B. AND C. SCHWARTZ. J. Clin. Investigation **11**: 817, 1932.
(9) BLOCK, C. E. AND F. FABER. Am. J. Dis. Child. **30**: 504, 1925.
TAYLOR, N. B., C. B. WELD, H. D. BRANION AND H. D. KAY. Canad. M. A. J. **24**: 736; **25**: 20, 1931.
(10) GRANT, J. H. B. AND F. L. GATES. J. Gen. Physiol. **36**: 635, 1924.
(11) OPPER, L. Arch. Path. **31**: 569, 1941.
(12) MEREDITH, W. C. A thesis presented to the Yale University School of Medicine, 1927.
(13) PAPPENHEIMER, A. M. AND S. L. WILENS. Am. J. Path. **11**: 73, 1935.

INFLUENCE OF BASAL FOREBRAIN AREAS ON THE ELECTROCORTICOGRAM

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Previous studies have shown that the electrical activity of the cortex may be modified by stimulation of the thalamus and some parts of the basal ganglia. Although the suggestion (Grinker and Serota, 1938) has been made that hypothalamic activity may also affect the cortex, incidental observations during the course of experiments directed primarily at the thalamus (Morison, Dempsey and Morison, 1941; and Morison and Dempsey, 1942) failed to demonstrate such a relation.

In the latter studies, exploration was carried out with single shock or relatively low frequency stimulation, anesthesia was relatively deep, and the effect on cortical areas presumed to have most intimate relation to the hypothalamus, notably gg. cinguli and proreus, was not investigated.

The present study was therefore carried out with the appropriate modification of the above details in the hope of establishing a more precise relationship between hypothalamus and the electrocorticogram. This objective was not achieved, but incidental information of some interest was collected.

METHODS. Cats anesthetized with nembutal were used. Ordinarily the level of anesthesia was such as to just abolish spontaneous movement, small additional amounts being added intravenously as necessary.

Records were taken from various cortical regions by means of small silver bipolar electrodes connected through 5 push-pull stage, condenser-coupled amplifiers to a multi-channel Grass ink-writer. Ordinarily the electrodes were placed upon the arm sensory area, the auditory area, the middle suprasylvian gyrus and in eight experiments the medial surfaces of gg. proreus and cinguli, appropriate removals of the opposite hemisphere being made to allow access to the latter two regions. In other experiments contralateral cortical regions were studied.

Arrangements for orienting the exploring electrodes and supplying stimulating pulses to them were similar to those used in preceding studies (Morison and Dempsey, 1942). At autopsy the brain was fixed in formalin for later identification of stimulating points by gross or frozen microscopic section.

RESULTS. 1. *Responses to single shocks.* The results of single shock activation of the hypothalamus may be dismissed in a word. They were essentially negative. Occasional secondary responses (Forbes and B. R. Morison, 1939) were encountered from the dorsal hypothalamus as described by Morison, Dempsey and Morison (1941), but the anesthesia was not ordinarily deep enough to favor their appearance routinely. The exposure of the gyri cinguli and proreus carried out in these experiments allowed these regions to be added to the now complete list of neo-cortical areas which exhibit this phenomenon.

The primary response recorded from proreus in a small number of cases by Morison and Dempsey (1942) was found routinely in these experiments when stimulating electrodes were in the anterior nuclear mass or anterior thalamic peduncle.

Effects on g. proreus from stimulation of mammillary bodies or the bundle of Vicq d'Azyr or on cinguli via the more complex path suggested by Le Gros Clark (1938) were not encountered.

2. *Responses to repetitive stimulation.* No effects which could unequivocally be attributed to stimulation of hypothalamic nuclei were seen. Two effects, one
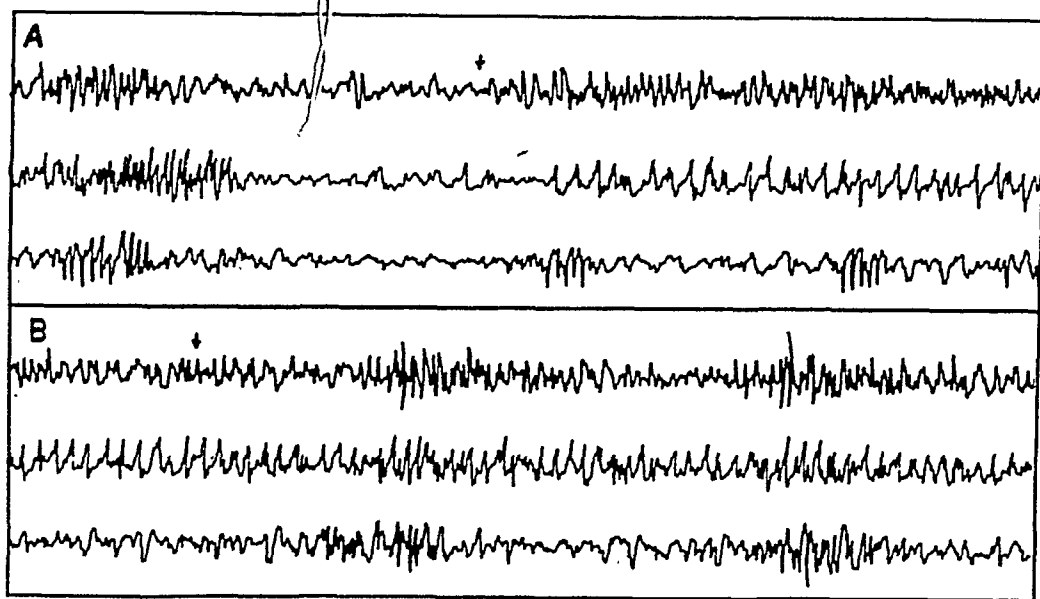
Fig. 1. Suppression of burst activity with increase of "projection" activity on anterior sigmoid gyrus, and 3 per sec. activity on anterior marginal. Note slow disappearance of effect after cessation of stimulation.

Upper tracing: anterior sigmoid gyrus; middle: anterior part of marginal gyrus; lower: middle suprasylvian. Arrows indicate the beginning and end of stimulation of the anterior part of the thalamic fasciculus of Forel, at 60 per second. Twenty-five seconds removed between A and B.

This and the succeeding records were taken at a paper speed of 15 mm. per second and reduced one half.

a generalized inhibition of the intermittent 6–10 cycle bursts normally characteristic of the nembutalized cat (figs. 1, 2 and 3), and the other a slow building up of 2–3 per sec. activity (figs. 2 and 4) were produced when the stimulating electrodes were in the hypothalamus, especially its more dorsal parts, but the excitable region extended laterally through the subthalamus, substantia nigra, etc., and at rostral levels even into the region of the amygdala and pallidum. As has already been shown (Dempsey and Morison, 1943), inhibition of the bursts, in nowise different from that under discussion, may also be obtained from the thalamus and was routinely present in these experiments. Equally good and in several instances more intense effects were encountered ventral to the thalamus in regions which failed to yield other responses typical of thalamic stimulation, but the wide

distribution laterally and rostro-caudally, i.e., from in front of the anterior to behind the posterior commissures, makes it unlikely that either the inhibition or the build up of slow activity belongs specifically to either thalamus or hypothalamus.

Distribution of points giving rise to the build up of slow wave activity was similar to that giving inhibition except that the thalamus was not importantly represented. The points therefore have a distribution similar to, though somewhat wider than, that found by Morison, Dempsey and Morison (1941) for those involved in the "secondary response."

Inhibition was best obtained at stimulus frequencies between 60 and 120 per second, but in favorable instances has been seen at frequencies as low as 15 and
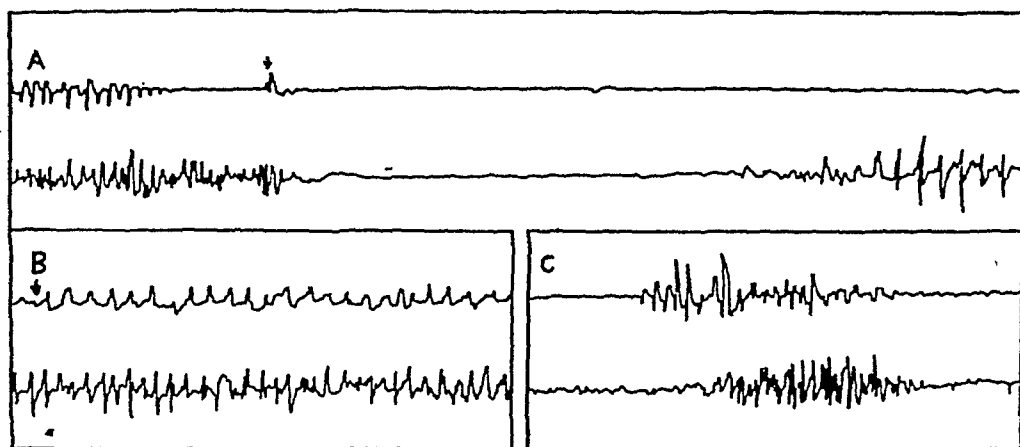
Fig. 2. Complete suppression of burst activity with gradual build up of 3 per second activity on anterior suprasylvian gyrus (lower tracing) and similar though slighter activity appearing as a rebound on the anterior sigmoid (upper tracing). Six seconds removed between A and B. The abnormal activity gradually declined over the succeeding 25 seconds until C the first completely normal burst on an essentially flat background appeared.

Stimulation at 60 per sec. in the region of fields of Forel at the level of the mammillary bodies.

as high as 600 per sec. For complete abolition of the bursts intensities about 2 to 4 times those necessary for producing the thalamic primary response (Dempsey and Morison, 1943) were usually required. In routine exploration therefore a frequency of 60 per sec. and an intensity approximating three times that of the threshold for the primary were adopted. The fact that autonomic responses such as pupillo-dilatation, cardiac acceleration, and respiratory changes were usually nowhere near maximal when the stimulus was applied to the hypothalamus justifies to some extent at least the employment of an intensity which may have contributed to the wide distribution of active points encountered.

Under these conditions, inhibition appeared as an increase in the normal interval between bursts and a reduction in their duration, most intense results being a complete abolition of this type of activity for stimulating periods of 1–3 minutes. In somewhat over half the cases the bursts returned either immediately or within a few seconds after cessation of stimulation (figs. 1 and 3).

In others a longer delay (up to 90 sec.) ensued. The occurrence of such "after discharge" of inhibition could not be correlated with the location of the stimulation point, or the level of anesthesia and only very generally with the intensity, frequency, or duration of stimulation. Not all cortical areas were equally depressed. In general somatic sensorimotor cortex gave the most striking results, the middle suprasylvian gyrus the least (fig. 1). In a few instances in which activity of both hemispheres was recorded, slight inhibition was found on the side opposite to the location of the stimulating electrodes, but this was never at all comparable to the homolateral effects.
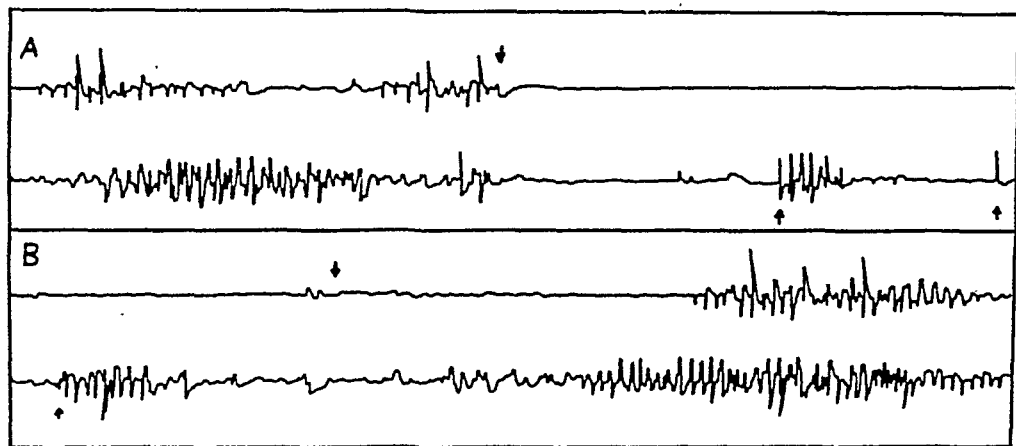
Fig. 3. Complete suppression of burst activity on anterior sigmoid (upper tracing) and superior sylvian (lower tracing) gyri with lack of effect on response to handclap in the latter. Downward arrows indicate beginning and end of stimulation just dorsal to mammillary bodies; upward arrows—handclaps. Ten seconds removed between A and B.
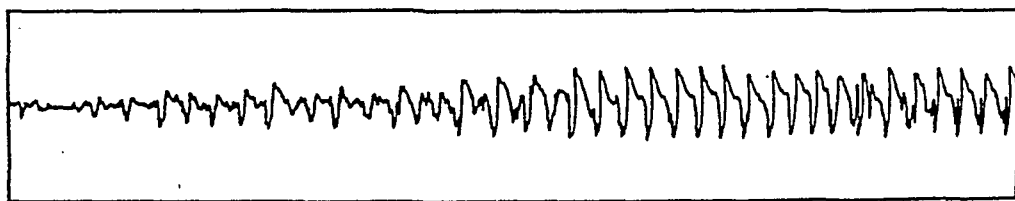
Fig. 4. Gradual build up of spike and wave formation in posterior sigmoid gyrus from stimulation in region of field H_2 of Forel at the level of the anterior border of the mammillary bodies. Stimulation commenced 2 sec. before beginning of record.

If the existing spontaneous cortical activity consisted of intermittent bursts separated by periods of little or no activity, suppression of the bursts resulted in a virtually flat record (figs. 2 and 3) which might later give way to a build up of 3 per second activity (fig. 2, lower tracing). Continuous spontaneous activity of the "projection" type (Dempsey and Morison, 1943) was, however, not suppressed and might even be increased both during stimulation and for some time afterwards (fig. 1, upper tracing). The somewhat random spike-like activity, similar to the secondary responses and attributable to a non-thalamic system (Morison, Dempsey and Morison, 1941), and more continuous slow-wave activity (2-3 per second), either "spontaneously" present in some experiments or

induced by stimulation of the areas described above, were likewise unaffected. Indeed stimulation of subthalamic areas often resulted in prolonged inhibition of bursts with a gradual superimposition of the 3 per sec. activity (figs. 2 and 4).

Responses produced by stimulation of a peripheral nerve (cf. Dempsey and Morison, 1943) or by a sharp handclap (fig. 4) were readily recorded in the presence of prolonged inhibition of bursts.

The building up of slow-wave activity referred to above was not so routinely encountered as the inhibitory phenomena. Very rarely such episodes have occurred "spontaneously" under the experimental conditions used here, and then only during the later stages of an experiment after the possibilities of non-specific damage to the cortex may have arisen.

In slightly over one-half of the present experimental series, prolonged stimulation in the regions already mentioned gave rise to episodes of slow-wave activity. The ease of elicitation was very variable even within a single experiment, and long rest periods were necessary in order to ensure repetition of the episodes in anything like their original form in the course of a series of identical stimuli. Not all cortical areas participated simultaneously in every episode, but at one time or another every area studied exhibited the phenomena.

Various patterns were found varying from a slight increase in rather nondescript and irregular slow waves to the definite wave and spike pattern evolved in the latter part of figure 4. The abnormalities rarely ceased coincidentally with the stimulus, but gradually disappeared over a period of from one to several minutes (fig. 2). Occasionally, after several inductions of the effect, normal activity failed to reappear at all and the experiment had to be discontinued.

Some of the most striking instances of the building up of cortical activity of low frequency were accompanied by increased respiration, and the question arose whether the change in the corticogram might be secondary to an alkalosis or other disturbance of homeostasis. Forced artificial respiration to an extent far greater than that consequent on stimulation failed, however, to induce significant changes in the record.

Perhaps the most conclusive evidence that the cortical changes were a direct result of the stimulus was derived from experiments in which the cortical activity was registered from both hemispheres. In such cases the abnormal waves usually appeared only on the stimulated side. In occasional instances bilateral effects were encountered, but these were always less marked on the side opposite to that stimulated.

DISCUSSION. The most plausible deduction that may be made from the distribution of points giving rise to inhibition of the cortical bursts is that this activity resides in fiber systems passing through the dorsal hypothalamus and subthalamus and regions immediately rostral and caudal thereto and represent lemniscal, lenticular, and possibly conjunctival systems on their way to the intralaminar nuclei concerned with the production of cortical bursts (Morison and Dempsey, 1942; Morison, Finley and Lothrop, 1943). Since stimulation of peripheral nerve (Dempsey, Morison and Morison, 1941), or the striatum (Dus-

ser de Barenne and McCulloch, 1938), have both been reported as inhibitory for at least some elements of the electrocorticogram such an interpretation is plausible enough. In our hands, at least, stimulation just ventral to the thalamus was ordinarily productive of more striking effects than was activation nearer to the origin of the fiber systems (for instance, the pallidum). This, however, may be explained by the greater concentration of fibers as they approach their endings in the thalamus.

It is important to emphasize that the inhibition affects the cortical bursts specifically and leaves the discrete responses evoked by sensory stimulation, the 2–3 per sec. activity induced by basilar stimulation, and two forms of spontaneous activity completely unmodified (p. 414). Not only does this suggest that the inhibition takes place in the thalamus and does not represent a generalized depression of the cortex, but it supports the general thesis that the electrocorticogram is a complex resultant of activity in relatively discrete systems separable morphologically and functionally from one another.

Little can be said about mechanisms underlying the production of the build-up activity. Apparently the thalamus is not importantly involved since the most active point was invariably ventral to it.

Because of the fact that these experiments failed to uncover any influence on the electrocorticogram when the stimulating electrodes were deep in the hypothalamus proper which was not also produced from more dorsal areas which extended both laterally and rostrocaudally, it was impossible to confirm Grinker and Serota in their view that the hypothalamus is importantly concerned with the electrical activity of the cortex. Ablation of the hypothalamus (Dempsey and Morison, unpublished), unless so extensive as to involve the blood supply to the thalamus, has also failed to suggest such a relation. The sort of change illustrated by Grinker and Serota as supporting their contention is more easily explained as a result of cutting the lemniscus (Cf. Dempsey and Morison, 1943).

SUMMARY

1. Single shock stimulation of the hypothalamus failed to produce significant changes in the electrical activity of any major cortical area.

2. Repetitive stimulation (15–600 per sec.) produced two significant effects: *a*, a generalized depression of the intermittent bursts of 5–10 per second frequency unaccompanied by suppression of various other types of cortical activity (figs. 1, 2, 3); *b*, a gradual and prolonged building up of slow-wave activity (2–3 per sec.).

3. Active points were found not only in the hypothalamus but in a relatively wide area lateral and posterior to it. Effect *a* but not *b* was also produced from the thalamus especially its medial part.

4. It appears that the inhibitory effect may be attributed to the activation of various fiber systems running through the dorsal hypothalamus to reach the areas of the thalamus shown by other studies to be important in controlling the intermittent cortical bursts.

REFERENCES

CLARK, W. E. LeG. The hypothalamus. Oliver and Boyd, Edinburgh, 1938.

DEMPSEY, E. W., R. S. MORISON AND B. R. MORISON. This Journal **131**: 718, 1941.

DEMPSEY, E. W. AND R. S. MORISON. This Journal **138**: 293, 1943.

DUSSER DE BARENNE, J. G. AND W. S. McCULLOCH. J. Neurophysiol. **1**: 364, 1938.

FORBES, A. AND B. R. MORISON. J. Neurophysiol. **2**: 112, 1939.

GRINKER, R. R. AND H. SEROTA. J. Neurophysiol. **1**: 573, 1938.

MORISON, R. S., E. W. DEMPSEY AND B. R. MORISON. This Journal **131**: 732, 1941.

MORISON, R. S. AND E. W. DEMPSEY. This Journal **135**: 281, 1942.

MORISON, R. S., K. H. FINLEY AND G. N. LOTHROP. In press.

STUDIES ON THE LINGUO-MAXILLARY REFLEX

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Gellhorn, Cortell and Carlson showed that the excitability of the autonomic centers in hypothalamus and medulla oblongata was greatly increased in anoxia whereas the reactivity of the somatic centers which was tested by stimulation of the same area was diminished. The experiments seemed to indicate a fundamental difference in the excitability of somatic and autonomic centers. The present experiments were designed to contribute further material to this problem and to include also conditions other than anoxia. They involve in contradistinction to our earlier work not direct but reflex stimulation of autonomic and somatic centers. Since the main vegetative centers are located in the medulla oblongata and adjacent parts of the pons (cf. Lim and collaborators for the sympathetic myelencephalic center, and Pitts, Magoun and Ranson for the respiratory center) it was decided to study the effect of changes in internal environment on respiration, blood pressure and a somatic reflex, the linguo-maxillary reflex (1, 2, 3, 5) whose center is located in the same section of the central nervous system. The action of anoxia, asphyxia, hypercapnia and hypoglycemia was investigated.

METHOD. The results are based on experiments performed on 48 cats anesthetized with 100 mgm./kgm. chloralose subcutaneously or 40 mgm./kgm. pentothal. Some additional experiments were performed on dogs with pentothal-chloralose anesthesia. Preparation of the digastric muscle and recording of its contraction was carried out as described by Blier and Kleitman. The blood pressure (Hürthle or Hg-manometer) and respiration were recorded in the usual manner. The gas mixtures prepared by means of flow meters were inhaled from Douglas bags. Condenser discharges at the rate of one per second were applied to the tongue in order to elicit the linguo-maxillary reflex. The blood sugar was determined by the Somogyi modification of the Shaffer-Hartman method.

RESULTS. 1. *Hypercapnia.* The fundamental difference in the reactivity of somatic and autonomic centers at the medullary levels is well illustrated by the study of the effects of 7.4 per cent carbon dioxide on blood pressure, respiration, and linguo-maxillary reflex. Whereas respiration increases and the blood pressure rises the linguo-maxillary reflex decreases. The effects were reversible and occurred uniformly in 23 animals.

The elimination of the chemo-receptors by bilateral vagotomy and denervation of the carotid sinus area did not change the type of reaction which is illustrated in table 2.

Hyperventilation during which the respiratory minute volume was increased

TABLE 1

Effect of restoring normal blood sugar levels upon the height of contraction of the linguo-maxillary reflex in insulin hypoglycemia cats

NO.	BLOOD SUGAR LEVEL BEFORE GLUCOSE INJECTION	AMOUNT OF GLUCOSE INJECTION	MAXIMAL CHANGE IN RESPONSE IN PER CENT OF CONTROL LEVEL	TIME AFTER INJECTION TO MAXIMAL RESPONSE	BLOOD SUGAR LEVEL AFTER INJECTION
	mgm. per cent	*cc. 7 per cent*	*per cent*	*min.*	*mgm. per cent*
1	48.3	15	+50	4	206
2	36.5	7	+300	9	93.4
3	38.2	25	+350	2	142
4	25.8	10	+250	4	129
5	34.4	10	+250	3	103
6	40.8	10	+400	2	93.5
7	59.1	15	+250	2	221
8	37.6	10	+50	3	137
9	47.3	15	+100	3	144
10	19.3	15	+400	3	108
11	16.4	20	+400	5	184

Effect of elevating blood sugar above normal levels

NO.					
1	104	15	No change		183
2	166	30	No change		236

TABLE 2

The effect of 7.4 per cent CO_2 on the linguo-maxillary reflex

A. Blood sugar normal (no insulin)

NO.	DURATION OF PERIOD OF INHALATION OF 7.4 PER CENT CO_2	MAXIMAL EFFECT IN PER CENT OF CONTROL	PERIOD OF RECOVERY TO 75 PER CENT CONTROL LEVEL AFTER REMOVAL OF CO_2
	min.	*per cent*	*min.*
1	10	−75	3
2	8	−100	3½
3	8	−90	2
4	14	−95	4
5	6	−100	3
6	10	−50	3
7	3	−90	1
8	6	−60	3
9	10	−50	2

B. Blood sugar reduced by insulin

Effect of inhalation of 7.4 per cent CO_2 upon the height of contraction of the linguo-maxillary reflex in the insulin hypoglycemia animal

NO.	DURATION OF CO_2 INHALATION	MAXIMAL CHANGE IN RESPONSE IN PER CENT OF CONTROL LEVEL	PERIOD OF RECOVERY TO 75 PER CENT CONTROL LEVEL	BLOOD SUGAR LEVEL
	min.	*per cent*	*min.*	*mgm. per cent*
1	1½	−100	5	36.5
2	2½	−75	4	63.4
3	2	−100	3	25.8
4	1½	−100	8	25.8
5	1½	−100	7	34.4
6	1½	−75	2½	40.8
7	3	−50	2	59.1
8	1½	−75	2	36.7

approximately 8 times led in 12 experiments on 3 animals to an increase of the linguo-maxillary reflex of 300 to 400 per cent. Here again a marked contrast existed between vegetative and somatic centers in as much as the excitability

of the autonomic centers decreased as indicated by the apnea following the period of hyperventilation and by a slight fall in blood pressure.

2. *Anoxia and asphyxia.* In 20 experiments in which 6.2 per cent oxygen was inhaled it was found in all but 3 that this degree of anoxia resulted either in a complete disappearance of the linguo-maxillary reflex or in considerable reductions. The effects were reversible in almost all instances within 10 minutes after readmission of air. In 3 instances this oxygen-nitrogen mixture failed to cause a change in the linguo-maxillary reflex. In all experiments an increased respiratory response and a rise in blood pressure accompanied this decrease of the linguo-maxillary reflex. Figure 1 illustrates a typical effect.
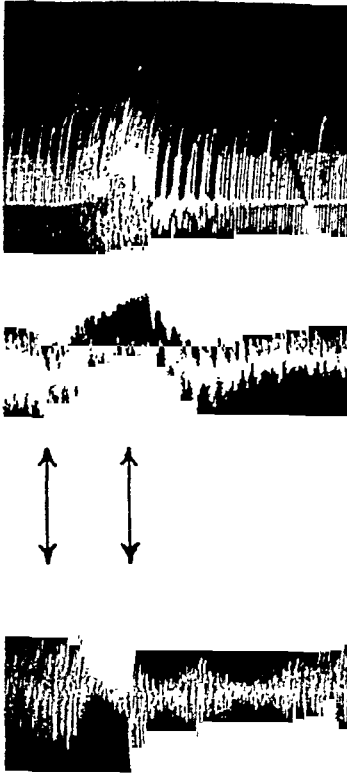
Fig. 1. Effect of 4.5 per cent O_2 for 2 min. on the linguo-maxillary reflex of the dog anesthetized with Na-amytal 40 mgm./kgm. intraperitoneally and chloralose 30 mgm./kgm. intravenously. Recordings from above downward: Respiration, blood pressure, linguo-maxillary reflex.

This experiment was repeated in 6 cats in which the carotid sinus area had been denervated and the vagi cut in order to determine the rôle of the chemoreceptors. It was found that under these conditions anoxia likewise either greatly reduces or completely abolishes the linguo-maxillary reflex. However, it was observed that the period of reduced reflex response was preceded by a temporary phase during which the reflex was increased. Since it is known from the investigations of Gellhorn and Lambert that elimination of the chemoreceptors leads to a fall in blood pressure in response to anoxia instead of the rise seen in normal animals it was thought that possibly the reduction in blood flow associated with a fall in blood pressure may account for the appearance of a temporary increase in the linguo-maxillary reflex response. It was found indeed that this reaction of the reflex could be reproduced in normal cats by administering 6.2 per cent oxygen while both carotid arteries were clamped.

Asphyxia induced by clamping of the trachea caused in 4 experiments a complete or nearly complete disappearance of the linguo-maxillary reflex while respiratory efforts were greatly increased and the blood pressure rose.

3. *Hypoglycemia.* Twelve experiments were performed upon six cats. The animals were rendered hypoglycemic by the intramuscular injection of from 4 to 8 units of insulin per kilogram one to two hours before the beginning of the experiment. A venous blood sample was obtained for sugar analysis. From 7 cc. to 25 cc. of 7 per cent glucose was then injected intravenously. Within from 2 to 9 minutes after the injection the height of contraction of the reflex increased from 50 per cent to 400 per cent above that recorded at the hypoglycemia level. A second venous blood sample was then taken for sugar analysis. Additional insulin was later injected and the experiment repeated. The results illustrated in table 1 showed that the injection of glucose restored the linguo-maxillary reflex although an increase of blood sugar above the normal value was without effect on the reflex response, as control experiments indicated.

These observations illustrate again the fundamental difference in the reactivity of somatic and vegetative centers in the same part of the central nervous system in as much as hypoglycemia is accompanied by an increased respiratory activity leading to a diminution in the carbon dioxide content of the arterial blood and a decrease in the carbon dioxide tension of the alveolar air (Domm and Gellhorn). Earlier studies of Gellhorn and collaborators (1938–1940) likewise demonstrated an increased reactivity of the vasomotor center in hypoglycemia.

Table 2 shows the effect of carbon dioxide on the linguo-maxillary reflex at normal and reduced blood sugar levels. It is evident that when the blood sugar had been reduced by insulin, carbon dioxide becomes more effective and causes a more rapid decline of the linguo-maxillary reflex than is observed at a normal blood sugar level. Moreover, the period of recovery is somewhat lengthened in the hypoglycemic experiments as compared to those performed without the use of insulin. These experiments are of interest in view of the fact that Gellhorn, Kiely and Hamilton observed that the pressor response elicited by carbon dioxide is inversely related to the blood sugar level. Apparently the effects of carbon dioxide are potentiated under condition of hypoglycemia. Whereas this potentiation results in a greatly increased blood pressure response to carbon dioxide, it causes a marked acceleration of the depressant action which carbon dioxide exerts on a somatic medullary reflex.

DISCUSSION. The experiments reported in this paper give clear evidence for the fact that alterations of the internal environment with respect to carbon dioxide, oxygen tension and blood sugar act in opposite manners on somatic and vegetative centers of the medulla. Whereas anoxia and hypercapnia cause a rise in blood pressure and an increased respiratory minute volume the linguo-maxillary reflex whose center is located in the brain stem is greatly depressed. Hypoglycemia which stimulates respiration and increases the reactivity of the vasomotor center to reflex-stimulation as well as to direct stimulation depresses

the linguo-maxillary reflex. When two of the factors such as carbon dioxide and low blood sugar are combined greatly augmented effects are obtained on both somatic and vegetative centers. Under these conditions the fundamental difference in the reactivity of autonomic[1] and somatic centers is retained.

The physiological significance of this fundamental difference is obvious. The increased respiratory activity under conditions of hypercapnia and anoxia tends to restore the internal environment and to minimize the effects which these disturbances in homeostasis would otherwise have on the somatic nervous system. This interpretation is applicable to the conditions of hypoglycemia because the increased excitability of the sympathetic myelencephalic center tends to restore the blood sugar through sympathetic-adrenalin discharges. Moreover, the increased respiration tends to raise the oxygen tension in the blood which is of importance since the effect of hypoglycemia on the brain can be eliminated by the inhalation of pure oxygen (Kessler and Gellhorn).[2]

SUMMARY

Somatic and autonomic reactions whose reflex center is located in the brain stem were studied under conditions of disturbances of the internal environment. It was found that hypercapnia while exciting respiration and blood pressure decreases the linguo-maxillary reflex. Hyperventilation resulting in a decreased excitability of the respiratory and vasomotor centers caused a marked increase in the intensity of the linguo-maxillary reflex. The fundamental difference in the reactivity of somatic and autonomic brain stem centers is further illustrated by experiments on anoxia and hypoglycemia. These conditions depress the linguo-maxillary reflex while they excite the autonomic medullary centers. If carbon dioxide is inhaled at various blood sugar levels it is found that the depressant action of carbon dioxide on the linguo-maxillary reflex is inversely related to the blood sugar level whereas its excitatory effect on the vasomotor centers increases with falling blood sugar. The physiological significance of these reactions for the protection of the brain and the maintenance of homeostasis is emphasized.

REFERENCES

(1) BLIER, Z. AND N. KLEITMAN. This Journal 94: 118, 1930.
(2) BONVALLET, M. AND B. MINZ. Compt. rend. Soc. de Biol. 128: 705, 1938.
(3) BRACE-ROUSSEN, H. CORDET AND H. LAUGIER. Compt. rend. Soc. de Biol. 89: 32, 1923.
(4) CHEN, M. P., R. K. S. LIM, S. C. WANG, AND C. L. YI. Chinese J. Physiol. 10: 445, 1936.

[1] The respiratory center innervating striated muscles is ordinarily not considered a part of the autonomic nervous system. However, it is likewise distinguished from the somatic nervous system by the persistence of its activity when voluntary control is eliminated. The present investigation showing that respiratory and vasomotor centers react similarly and in contrast to closely located somatic centers, suggests the classification of the respiratory center as a part of the autonomic system.

[2] For further discussion cf. Gellhorn, 1943.

(5) Cordet, H. and H. Laugier. Compt. rend. Soc. de Biol. 86: 529, 1922.

(6) Domm, S. and E. Gellhorn. Arch. Neurol. and Psychiat. 43: 726, 1940.

(7) Gellhorn, E. 'Autonomic regulations. New York, 1943.

(8) Gellhorn, E., R. Cortell and H. B. Carlson. This Journal 135: 641, 1942.

(9) Gellhorn, E., R. C. Ingraham and L. Moldavsky. J. Neurophysiol. 1: 301, 1938.

(10) Gellhorn, E. and M. Kessler. This Journal 136: 1, 1942.

(11) Gellhorn, E., W. F. Kiely, and S. L. Hamilton. This Journal 130: 256, 1940.

(12) Gellhorn, E. and E. H. Lambert. Univ. of Ill. Press, Urbana, 1939.

(13) Ingraham, R. C. and E. Gellhorn. Proc. Soc. Exper. Biol. and Med. 40: 315, 1939.

(14) Pitts, R. F., H. W. Magoun and S. W. Ranson. This Journal 127: 654, 1939.

(15) Yesinick, L. and E. Gellhorn. This Journal 128: 185, 1939.

OBSERVATIONS ON THE EFFECT OF CERTAIN DRUGS ON THE SMALL BLOOD VESSELS OF THE RABBIT EAR BEFORE AND AFTER DENERVATION[1]

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The exact nature of the rôle of sympathetic innervation in the reactions of blood vessels has long been a subject of interest to physiologists, neurologists and clinicians (1–7). Much work has been done on this question and many important observations have been made. There are still certain aspects of the problem, however, which remain either vague or controversial. Most of the studies made previously have been carried out with the experimental animal under general anesthesia and, in many instances, changes of the caliber of the blood vessels have been inferred by measurements of temperature, blood pressure or alterations of blood flow. The objects of the present study were 1, to observe directly the reactions of the small blood vessels of the ear of the rabbit to certain drugs (epinephrine hydrochloride, ephedrine, ergotoxine and pitressin); 2, to observe the reactions to these drugs of the minute vessels in the innervated and in the denervated ear; 3, to determine the rôle played by the auricular nerves in motor responses of the minute blood vessels of the ear by direct observations of the reactions of these blood vessels to stimulation of the nerves; 4, to correlate the reactions of the blood vessels with evidences at necropsy of regeneration of nerves, and 5, by these means to determine the function performed by the nerves which supply these blood vessels. For this study we have found a modification of the Clark transparent chamber inserted into the ear of the rabbit satisfactory.

METHOD. The transparent chamber we have used is similar to that which has been described by Ebert, Florey and Pullinger (8). This type of window is fashioned on a lathe from a bar of lucite. The advantages over the original one used by Sandison (9) are 1, that the new type does not warp; 2, that it is more transparent than the original type, and 3, that it may be used repeatedly if desired.

Healthy albino rabbits, weighing between 2 and 4 kgm., were used for this study. All surgical procedures, but not the subsequent observations, were carried out with the rabbit under anesthesia produced by pentobarbital sodium (25 to 35 mgm. per kilogram of body weight). The window was inserted at the junction of the middle and distal thirds of the ear between the marginal vein and the central vessels. Space does not permit a detailed description of the operative procedure for insertion of the window.

Most of these windows were fully vascularized at the end of four weeks. Ob-

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[1] Abridgment of thesis submitted by Doctor Levinson to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of M.S. in Medicine.

servations on the reaction of the blood vessels to drugs were made from four to six weeks after the insertion of the window. Particular attention was given to the response of the arterioles to injections of epinephrine, ephedrine, pitressin and ergotoxine. Constriction of the arterioles was graded on the basis of 1 to 4; 1 representing slight constriction; 2, moderate constriction (lumen diminished 50 per cent); 3, fairly marked constriction (still slight evidence of a lumen remaining); 4, marked constriction (complete disappearance of the arteriolar lumen). The temperature of the room registered from 21 to 26.5°C. during these studies. Injections of the drugs were made into the marginal vein of the ear opposite the one being observed. If there was constriction of the arteriole on insertion of the needle, we waited until the lumen had returned to its original caliber before starting injection of the drug. Control injections of isotonic saline solution did not produce any response in the vessels. Observations were carried out by means of a compound microscope at a magnification of 200. No attempt was made to measure the caliber of the vessels, since slight movement of the rabbit and the evanescence of some of the reactions made accurate measurement of the vessels difficult. In addition to direct observation, photographs were made of typical responses of the minute vessels to the various drugs. The observations were made on a series of nine animals. Repeated observations were made on each animal with each drug. The most consistent reaction produced by repeated doses of each drug was determined for each animal.

The epinephrine used was a stock solution of 1:1,000. Further dilutions were made with physiologic salt solution. The smallest dose of epinephrine to which any animal reacted was 0.05 cc. of 1:1,000,000 solution. With this dose as a starting point, the dose was increased by increments of 0.05 cc. until a grade 4 response was obtained. It was not necessary to consider the weight of the rabbit since the same doses of epinephrine were used for each animal before and after denervation.

To test the effect of ephedrine, crystals of ephedrine (Burroughs, Wellcome and Co.) were dissolved in physiologic saline solution in a strength of 10 mgm. per cubic centimeter. Doses of 1 mgm. per kilogram of body weight were administered at intervals of ten to fifteen minutes until there was a definite diminution of response but the number of successive doses never exceeded four.

Dilutions of pitressin (Parke, Davis & Co.) with physiologic saline solution to a strength of 2.0 pressor units per cubic centimeter were made just prior to injection and given in doses of 0.1 pressor unit per kilogram of body weight; the same procedure was carried out as with ephedrine.

To test the effect of ergotoxine, 5 mgm. of powdered ergotoxine alkaloid (Burroughs, Wellcome and Co.) was dissolved in 0.5 cc. of absolute alcohol, then made up to a volume of 5 cc. with distilled water and given in doses of 0.5 mgm. per rabbit. Administration of the dose of ergotoxine was preceded in each instance by administration of the smallest dose of epinephrine that produced a response of grade 4. Also in each instance administration of the dose of ergotoxine was followed by administration of a similar dose of epinephrine in order to observe any alteration of the response to epinephrine caused by ergotoxine.

After the foregoing studies had been completed, denervation of the ear was done. This consisted in removing pieces 15 to 20 mm. long of the dorsal and great auricular nerves of the ear in which the window had been placed. On the same side the peripheral portion of the cervical sympathetic chain, together with the superior cervical ganglion, was avulsed. The vessels were observed immediately after this operation and at intervals of five, ten, fourteen, twenty-one days and so forth after denervation. Studies of the action of drugs were also carried out at the intervals just mentioned.

Necropsies were performed on the animals and the condition of the auricular and cervical sympathetic nerves was noted.

Similar observations were made on one rabbit on which the entire operative procedure for denervation was carried out, except that the nerves were not cut. This animal served as a control on the effects of the operative procedures.
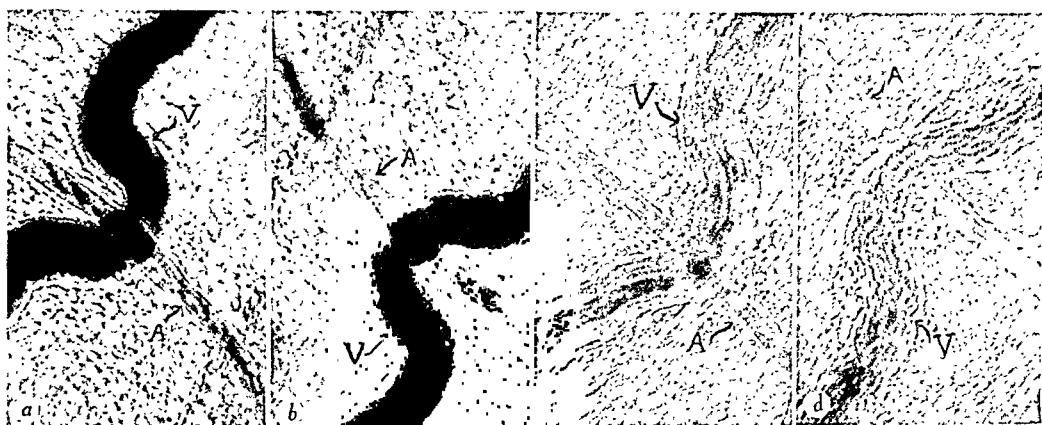
Fig. 1. Effect of denervation on the response of small blood vessels to administration of epinephrine ($\times 200$). *a*, appearance of arteriole (A) and venule (V) in the innervated ear; *b*, appearance of same vessels as seen in *a* thirty seconds after 0.2 cc. of 1:1,000,000 solution of epinephrine had been given; *c*, appearance of same vessels as seen in *a* ten days after denervation of the ear; *d*, appearance of same vessels as seen in *c* after 0.2 cc. of 1:1,000,000 solution of epinephrine had been given.

RESULTS. *Epinephrine.* The reaction of the vessels in the window of the innervated ear to epinephrine was usually a constriction of the arterioles within twenty seconds after the injection (fig. 1*a* and *b*). It was observed that not all the arterioles in the window responded equally to the same dose of epinephrine. At times, after administration of small doses of epinephrine, there were noted at irregular intervals along the course of an arteriole constrictions which gave the vessel the appearance of a string of sausages. This phenomenon tends to support the smooth muscle motor unit concept of Fulton and Lutz (10). The maximal constriction lasted usually from thirty seconds to one minute. Dilatation was frequently observed for a few seconds after relaxation of the vessel. There was a slight narrowing of the lumina of the venules accompanying the arteriolar constriction. Independent constriction of the capillaries was not observed. So long as any blood was flowing through the arterioles the capillaries remained open. It was only when arteriolar flow was shut off completely that the capillary lumina seemed to disappear.

After denervation (fig. 1c and d) it was observed in all instances that the does of epinephrine required to produce the grade 4 response was definitely less than for the vessels in the innervated ear.   In most instances there was only a slight difference in the size of the necessary dose but in a few cases the difference was very great.   This altered response was usually present when the animal was first studied at five days after denervation.   It was invariably present at ten days after denervation.   In addition the response to epinephrine usually occurred more slowly and lasted longer than in the innervated ear.   This increased response to epinephrine disappeared in a few cases after about four weeks following denervation.

Ephedrine.   In the animals that had innervated ears ephedrine had the same general effect as epinephrine.   However, the effect of ephedrine was more inconsistent than that of epinephrine and usually did not appear until about a minute after injection.   The maximal arteriolar constriction usually lasted from three to four minutes.   In each case the vessels showed either a diminished or a negative response to repeated doses.

The response of the vessels of the denervated ear to ephedrine was not greatly different from that of the vessels of the innervated ear.   In a few animals after denervation of the ear more ephedrine had to be given before the vessels became refractory to the action of the drug than had been necessary when the nerves were intact.

Pitressin.   Pitressin had the most powerful vasoconstrictor action on the vessels of the ear of any of the drugs used.   Its effect, like that of epinephrine, was chiefly on the arterioles.   Independent capillary constriction was not observed.   Abell and Page (11) and Barrett (12) have also reported that they did not observe independent capillary constriction after injections of pitressin.   The arteriolar constriction usually occurred within twenty to thirty seconds after injection.   The maximal effect in most cases lasted from three to ten minutes.   As with ephedrine, the effect on the arterioles of repeated doses either became diminished or was negative.

In the denervated ear the response was much the same as in the innervated ear with one exception.   In those animals in which there had been diminished response or absence of response of the auricular vessels to repeated doses, the same vascular response occurred, after denervations, with each repeated dose (fig. 2).

Ergotoxine.   Almost without exception in animals that had innervated ears ergotoxine produced a definite constriction of the arterioles which usually began about thirty seconds after injection.   The maximal constriction lasted from two to fifteen minutes.   This was then followed by a marked dilatation of the capillaries and a moderate dilatation of the venules.   Temporary arteriolar dilatation also was observed occasionally after the period of constriction.   The vessels of the innervated ear after injection of a dose of epinephrine which followed the administration of ergotoxine showed no effect whatever or a definitely diminished effect.   In those cases in which there was some constriction of the arterioles when administration of epinephrine followed that of ergotoxine, there was not any effect on the capillaries.

The response of the vessels of the denervated ear to ergotoxine was the same as that of the vessels of the innervated ear with two exceptions. First, ergotoxine produced a definite constriction of the arterioles in those few animals
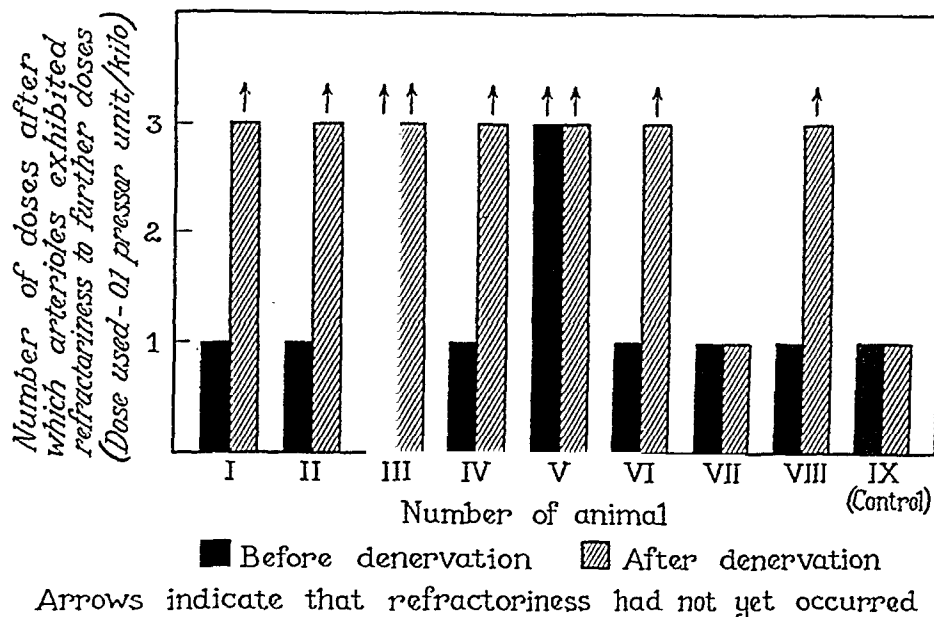
Fig. 2. Number of doses of pitressin required to produce refractoriness of arterioles to further doses. Effect before and after denervation.
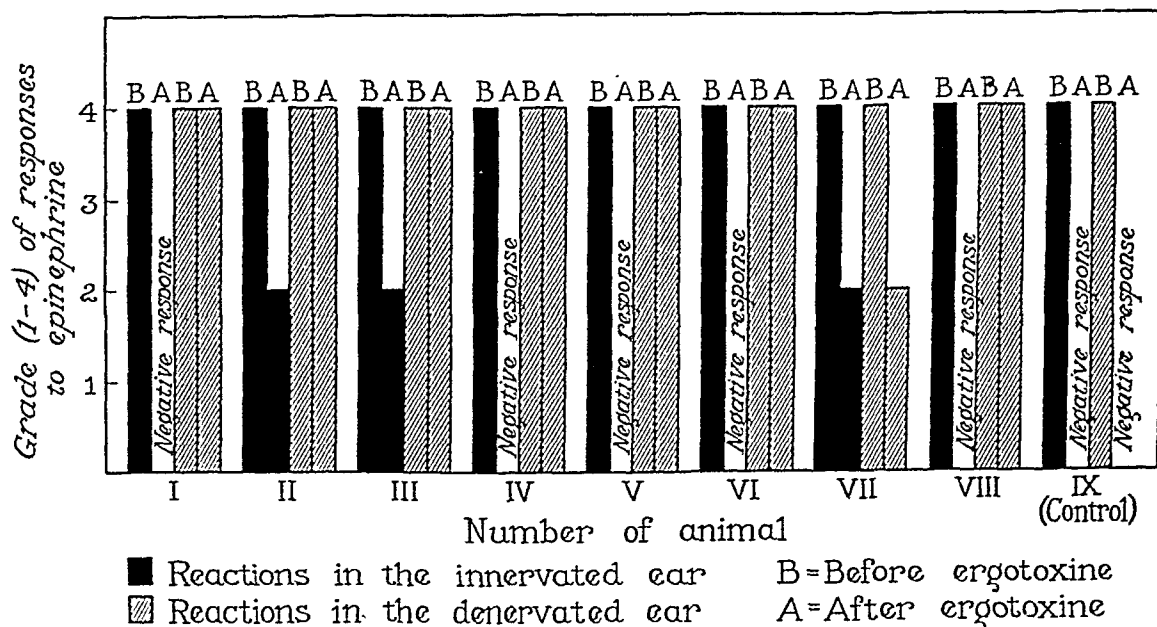
Fig. 3. Effect of denervation on the inhibitory action of ergotoxine

which had not shown any response of the arterioles to ergotoxine before denervation. Second, there was an alteration of the inhibitory action of ergotoxine, occurring usually between the fifth and the fourteenth day after denervation (fig. 3). During this period except in one case ergotoxine apparently had little

or no effect on the action of epinephrine. However, in those animals observed for more than two weeks the effect of ergotoxine became the same as it had been before denervation.

Effects of stimulation of the peripheral ends of the severed nerves. At the end of the first eight days of growth Clark, Clark and Williams (13) demonstrated nerves in the tissue which had grown into the chamber inserted into a rabbit's ear. It has also been shown (20) that stimulation of the peripheral end of the cervical sympathetic chain results in a swelling of the endothelial cells of the capillaries in this chamber. Meltzer and Meltzer (3) found that in the majority of cases, the third cervical nerve carries the vasomotor fibers for the blood vessels of the rabbit's entire ear, except for a comparatively small region around the lower two-thirds of the central artery, which is supplied by the cervical sympathetic nerves. In each of the animals we have studied except the control animal 9, we have recorded the changes of the vessels in the chamber subsequent to stimulation of the peripheral ends of the severed auricular and cervical sympathetic nerves.

Stimulation of the peripheral end of the cut cervical sympathetic chain invariably produced marked constriction of the arterioles in the chamber (table 1). This usually began about twenty seconds after the beginning of stimulation and lasted for one to two minutes after stimulation had ceased. Slight narrowing of the venules was also observed. No definite independent capillary constriction was observed. The disappearance of the capillary lumen seemed to be secondary to marked constriction of the lumen of the supplying arteriole.

Stimulation of the peripheral end of either the dorsal or the great auricular nerve did not produce a consistent response (table 1). In one animal no response was observed on stimulation of either of these nerves. In other animals a response was observed on stimulation of one nerve but not on stimulation of the other. In only one animal was a response obtained by stimulation of each of these nerves. When response to stimulation of these nerves did occur, the great auricular nerve produced a more marked effect than the dorsal auricular nerve. As with the cervical sympathetic chain no active constriction of the capillaries was noted after stimulation of these nerves.

Observations on the status of the vessels after denervation. Immediately after the sectioning of the auricular and cervical sympathetic nerves there appeared to be a definite dilatation of the arterioles, venules and capillaries in the chambers of all of the animals studied. (Animals had been anesthetized for some time when this was done.) When the animals were observed five days after denervation, it was noted in all instances that the arterioles were definitely diminished in caliber, although in some animals the reduction was only slight. The constriction seemed to be independent of environmental temperature or the degree of quietness of the animal and it seemed to persist. In two animals in which the hypersensitivity to epinephrine had disappeared at the end of about four weeks after denervation, there was concomitantly a return of the arterioles to their original caliber.

Postmortem findings on the sectioned nerves. There was not any evidence of

regeneration of the cervical sympathetic chain in any animal in the series.   In only one animal was there found gross evidence of regeneration of the auricular nerves.   In this rabbit there was an actual bridging of the great auricular nerve at the point where it had been sectioned forty-four days previously.   The whole nerve appeared larger than the one on the intact side and there were bulbous enlargements along its course.   In this animal there was a nodular swelling

TABLE 1

Effect of stimulation of nerves supplying the ear and condition of severed nerves at necropsy

RABBIT	EFFECT OF STIMULATION OF VARIOUS NERVES ON CONSTRICTION, GRADE, OF VESSELS				GROSS EVIDENCE OF REGENERATION OF NERVE AT NECROPSY	DAYS AFTER OPERATION
	Vessels	Dorsal auricular	Great auricular	Cervical*		
1	Arterioles	0	4	Not ob-	None	30
	Capillaries	0	0	served		
2	Arterioles	0	2	2	None	43
	Capillaries	0	0	0		
3	Arterioles	0	3	4	None	37
	Capillaries	0	0	0		
4	Arterioles	2	4	3	Great auricular	44
	Capillaries	0	0	0	nerve	
5	Arterioles	2	0	4	Not observed	
	Capillaries	0	0	0		
6	Arterioles	3	0	4	None	10
	Capillaries	0	0	0		
7	Arterioles	0	0	4	None	15
	Capillaries	0	0	0		
8	Arterioles	0	4	2	None	14
	Capillaries	0	0	0		

* Pupil dilated.

of the dorsal auricular nerve at the proximal end of the distal segment of the cut nerve.   The proximal segment could not be found.   There was no evidence of regeneration of this nerve.

COMMENT.   The increased sensitivity to epinephrine and the return of "vascular tone" after denervation remain enigmas.   It seems, however, that these phenomena are associated with the interruption of the sympathetic nerves. It is very interesting that the increased sensitivity to epinephrine and the diminished caliber of the vessels in a few animals disappeared when according to the work of others (14, 15) it might be suspected that regeneration of nerves .

had taken place. However, we were able to demonstrate regeneration anatomically in only one animal, although there was evidence of return of function in the others.

We have no adequate answer as to why the blood vessels exhibited increased sensitivity to drugs after interruption of their sympathetic nerve supply. Rosenblueth and Morison (16) suggested that denervation increases the permeability of smooth muscle cells to epinephrine. The slowness of the response and the increased duration of the effect of epinephrine after denervation would tend to indicate some alteration of a hypothetical receptive substance rather than a change of permeability. Ascroft (5) expressed the opinion that there is not any reason to doubt that sensitization developing after denervation is due to change of the peripheral structures. Grant (2) suggested that there must be some as yet unidentified pressor substance in the circulation which would account for the return of "vascular tone." This still would necessarily imply an altered reactivity of the blood vessel to this pressor substance.

The altered effect of ergotoxine after denervation lends support to the idea of a change of some mechanism associated with the blood vessel. Cannon and Rosenblueth (17) have indicated that the blocking effect of ergotoxine occurs between the "receptive substance" and the "specific response." If some alteration of this receptive substance had occurred after denervation, making the substance more responsive to epinephrine, would it not then be more difficult for a given dose of ergotoxine to inhibit this response after denervation? This is apparently just what occurred.

The fact that these reactions have been the same, on the whole, as those demonstrated by others (9, 11, 18–20) using different methods justifies the use of the tissue grown into the chamber inserted into the rabbit's ear as a physiologic experimental unit. The fact that the surgical procedures necessary to prepare the rabbits for our observations—procedures which were performed with the animals under anesthesia—preceded the observations by several weeks and that therefore the observations have been carried out successfully in the absence of anesthesia obviates one of the chief criticisms of this type of experiment, since the reactions are those to the drug administered and not those to the drug plus an anesthetic agent.

The problem of tachyphylaxis affords opportunity for much speculation. Local immunity (21), circulating antibodies (22) and cardiac depression (23) have been suggested as responsible for the phenomenon of tachyphylaxis. Rietschel (24) expressed the opinion that tachyphylaxis rests on a conversion or change of the vegetative nervous system. The fact that we have found in our experiments on ephedrine and pitressin that tachyphylaxis is produced with greater difficulty after denervation than before it lends support to the conception that the sympathetic nervous system is involved in this phenomenon but in what manner is not known.

In our experiments we have never observed definite, independent capillary contraction. We have not given attention to the swelling of the endothelial cells which Sanders, Ebert and Florey observed.

Stimulation of the peripheral end of the severed cervical sympathetic chain consistently produced a definite constriction of the arterioles in the chamber, while the results after stimulation of either auricular nerve were variable. It has been stated from the work of Meltzer and Meltzer that the vasomotor supply of the central artery in most instances comes largely from the cervical sympathetic chain. Since most of the blood vessels in the chamber usually grow in from the central artery (25) it seems logical to assume that these new vessels should be supplied by the same nerve fibers which supply the central artery. This might explain the consistent results obtained by stimulation of the cervical sympathetic chain. In those instances in which stimulation of the auricular nerves resulted in definite constriction of the arterioles, it can likewise be concluded that the arterioles received at least a part of their vasomotor supply through the auricular nerves and it may be inferred that the central artery did likewise.

SUMMARY AND CONCLUSIONS

A study of the reactions of the minute blood vessels seen in the transparent chambers inserted in the ears of albino rabbits has been presented. The response of these blood vessels to epinephrine, ephedrine, pitressin and ergotoxine has been recorded for innervated and denervated ears. Observations were also made on the effect of stimulation of the auricular and cervical sympathetic nerves on these blood vessels. Each animal was grossly examined at necropsy for evidence of regeneration of nerves.

The following conclusions were drawn:

1. The tissue grown into a transparent chamber inserted in the rabbit's ear is entirely suitable for physiologic studies.

2. The response to epinephrine was a consistent constriction of the arterioles accompanied by slight narrowing of the venules.

3. Ephedrine, in general, produced the same response as epinephrine. There was evidence of refractoriness to repeated doses of ephedrine.

4. Pitressin in the dose used (0.1 pressor unit per kilogram of body weight) had the most pronounced vasoconstrictor action. There was, as with ephedrine, refractoriness to repeated doses of pitressin.

5. Ergotoxine blocked the vasoconstrictor action of epinephrine.

6. Stimulation of the cervical sympathetic chain produced a constriction of the arterioles in the window more consistently than did stimulation of the dorsal or great auricular nerves.

7. The denervated vessels in the chamber became hypersensitive to epinephrine and regained their "tone" after denervation. Suggestions have been made as to the mechanism involved.

8. The blocking action of ergotoxine and the phenomenon of tachyphylaxis are apparently somewhat altered by denervation.

REFERENCES

(1) LeCompte, P. M. This Journal 135: 43, 1941.
(2) Grant, R. T. Clin. Sc. 2: 1, 1935.

(3) MELTZER, S. J. AND C. MELTZER. This Journal 9: 57, 1903.

(4) HERRICK, J. F., H. E. ESSEX AND E. J. BALDES. This Journal 103: 592, 1933.

(5) ASCROFT, P. B. Brit. J. Surg. 24: 787, 1937.

(6) FATHEREE, T. J., A. W. ADSON AND E. V. ALLEN. Surgery 7: 75, 1940.

(7) WHITE, J. C., A. M. OKELBERRY AND G. P. WHITELAW. Arch. Neurol. and Psychiat. 36: 1251, 1936.

(8) EBERT, R. H., H. W. FLOREY AND B. D. PULLINGER. J. Path. and Bact. 48: 79, 1939.

(9) SANDISON, J. C. Anat. Rec. 54: 105, 1932.

(10) FULTON, G. P. AND B. R. LUTZ. This Journal 135: 531, 1942.

(11) ABELL, R. G. AND I. H. PAGE. J. Exper. Med. 75: 305, 1942.

(12) BARRETT, R. H. Unpublished data.

(13) CLARK, E. R., E. L. CLARK AND R. G. WILLIAMS. Am. J. Anat. 55: 47, 1934.

(14) LEE, F. C. The regeneration of sympathetic nerve-fibers. Baltimore, Williams & Wilkins Co., 1930, 819 pp.

(15) LANGLEY, J. N. J. Physiol. 22: 215, 1897.

(16) ROSENBLUETH, A. AND R. S. MORISON. This Journal 109: 209, 1934.

(17) CANNON, W. B. AND A. ROSENBLUETH. Autonomic neuro-effector systems. New York, The Macmillan Co., 1937, 229 pp.

(18) WILSON, H. C. J. Pharmacol. and Exper. Therap. 56: 97, 1936.

(19) DALE, H. H. AND A. N. RICHARDS. J. Physiol. 52: 110, 1918–1919.

(20) SANDERS, A. G., R. H. EBERT AND H. W. FLOREY. Quart. J. Exper. Physiol. 30: 281, 1940.

(21) CHAMPY, C. AND E. GLEY. Quoted by RIETSCHEL.

(22) PAGE, I. H. AND O. M. HELMER. J. Exper. Med. 71: 495, 1940.

(23) GEILING, E. M. K. AND D. CAMPBELL. J. Pharmacol. and Exper. Therap. 29: 449, 1926.

(24) RIETSCHEL, H. G. Arch. f. exper. Path. u. Pharmakol. 193: 454, 1939.

(25) ABELL, R. G. AND E. R. CLARK. Anat. Rec. 53: 121, 1932.

THE ELECTRIC POTENTIALS OF THE HUMAN SMALL INTESTINE[1]

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Rosenblueth and his collaborators investigated the electric potentials of smooth muscle. They utilized the nictitating membranes, pilomotor muscles, uterus and urinary bladders of cats. Early studies by Rosenblueth and Rioch (1) in 1933 revealed the electrical activity of smooth muscle divisible into two phases—an initial potential which precedes contraction and a delayed potential simultaneous with the contraction. In a later publication Lambert and Rosenbleuth (2) in 1935 were able to subdivide still further the potentials of isolated preparations. Three phases were delimited—potential I, the fast, initial monophasic potential which precedes contraction; potential II, larger, slower mono- or diphasic potentials, either preceding or occurring simultaneously with the onset of contraction; potential III, consisting of two parts, IIIb, a slow potential lasting for the duration of the contraction, and IIIa, superimposed slow potentials, of shorter duration. In a subsequent publication Rosenblueth, Davis and Rempel (3) in 1936 demonstrated that potential I is not a propagated disturbance, is probably produced by the chemical mediator, and that the electric potential of neighboring cells may add in series.

Berkson (4), in 1933, using a string galvanometer, recorded relatively slow potentials arising from the small intestine of experimental animals. These potentials could not be correlated with contractions, and he considered them artefacts. Bozler (5) in 1938 studied the action potentials of strips of small intestine of experimental animals. During relaxation he could detect no electrical activity. Stimulation produced repetitive discharges and on rhythmic movements bursts of impulses appeared. Later Bozler (6) in 1939 isolated loops of small bowel in animals and found each contraction accompanied by repetitive discharges, each pendular movement accompanied by a discharge of 3 to 15 spikes, and in the guinea pig a characteristic frequency of 1 or sometimes several spikes occurring at an average of every 1 to 1.2 seconds during resting phases.

The experiments described in this paper were conducted in order to determine whether or not action potentials could be elicited from the intact human intestine and, if so, whether these potentials could be correlated with mechanical evidence of contraction, and with the electric potentials of smooth muscle as determined in animal experiments.

METHODS. A total of five patients was studied. Four were normals and one had a well-established ileostomy.

For recording from the patient with the ileostomy, Ag·AgCl electrodes were

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placed approximately $\frac{1}{2}$ cm. apart, directly on the mucosal surface, and parallel to the long axis of the ileum. Contractions were observed instead of being measured mechanically.

The normal patients were studied by intubation. The tube was single-lumened enclosing two no. 32 enamelled, copper wires. Electrode bars were soldered to the proximal ends of the wires.

The distal end of the tube was perforated to permit inflation of the balloon. The wires were brought through the end of the tube and electrodes of solder affixed, filed smooth to a diameter of approximately 4 mm. The lengths of protruding wire were adjusted to 3 and 5 cm. A solid, rubber-covered tip was inserted and fixed in place. A balloon of condom rubber, approximately 6 cm. long and adjusted to hold 50 cc. of air without stretching, was tied in place.

The electrodes were then sewed 2 cm. apart onto $\frac{1}{2}$ inch cotton tape. The tape was adjusted and tied so that inflation of the balloon kept it taut. This assured as nearly a perfect contact with the intestinal wall as possible during the studies. All connections were tested to prevent loss of air.

The tube was introduced into the small intestine by the method of Miller and Abbott (7) and under fluoroscopic guidance it was permitted to progress until it had passed the ligament of Treitz. Since the tube was stiffened by the wires, in most instances, it progressed very little from this point. A ground electrode was attached to the skin of the abdomen over the region of the tip of the tube. All recordings were made by a 3 channel, condenser coupled amplifier with ink writers (Grass).

The proximal end of the tube was connected to a tambour with a vertical-writing pen. The membrane of the tambour was selected to be relatively resistant so that its excursions did not interfere with the writing pens. The balloon was inflated with 50 cc. of air through a T-tube. The air was measured at intervals throughout the experiment to assure inflation and detect air leaks. The pen was aligned with those of the Grass oscillograph and continuous electrical and manometric records were made for periods of 2 to 3 hours.

RESULTS. In the patient with the ileostomy, whenever a contraction was visually apparent an electrical disturbance was evident. This disturbance consisted of fast potentials at the inception, followed quickly by slow activity. The base line shift indicated a very slow potential similar to IIIb described by Rosenblueth and his collaborators. Superimposed upon this potential was other slow activity but intermediate in frequency between the initial spikes and base line sway. This base line shift was either mono- or diphasic (fig. 1).

With the solder disc electrodes attached to the balloon in the jejunum a similar type of activity was obtained. In these records, however, the electrocardiogram appeared constantly (fig. 2). The rapid initial potentials are not as clearly evident in records taken in this manner and the faster potentials are frequently dispersed for a considerable distance along the record. This may well be due to the larger diameter of the solder electrodes as compared with the Ag·AgCl electrodes and also to the greater distance between the former. It is conceivable that initial potentials from more neighboring fibres are available for recording.

A polyphasic nature of the base line shift is more easily discernible in records obtained with the solder disc electrodes.

Correlation of the electric potentials with the mechanogram—a tambour in continuity with the tube and inflated balloon—reveals that the electrical activity is evidenced for an appreciable time before mechanical contraction is recorded (fig. 2). This time interval was constant for a single experiment and often was as much as 1.5 to 2 seconds.

The electrical activity not only preceded but usually did not persist for the duration of the mechanical evidence. Indeed, the onset of another contraction could at times be discerned electrically before the mechanogram had reached the base line. When the record is obtained at a faster speed, the difference
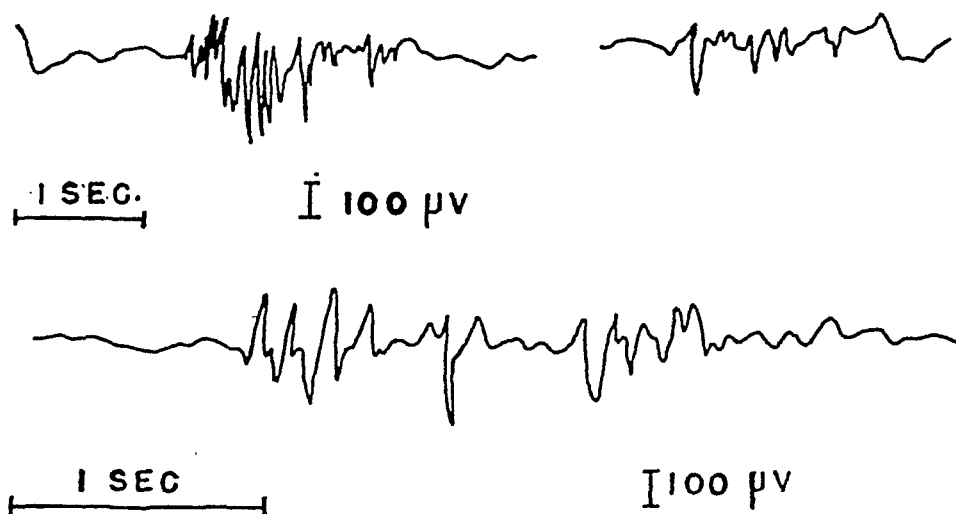
Fig. 1. Records obtained by means of Ag·AgCl electrodes from mucosal surface of small intestine exposed by old ileostomy. Upper two tracings at slower speed than lower tracing. Each record taken during contraction. Onset of activity is with fast frequency spikes followed by slow base line shift with superimposed intermediate activity.

between the three types of activity—the initial rapid spikes, the base line shift and the intermediate slow waves—is well exemplified (fig. 2).

DISCUSSION. Electrical potentials were obtained from the intact human intestine by means of electrodes introduced via intubation. The activity thus obtained shows considerable similarity to that described for isolated preparations of smooth muscle. That discrepancies should be present is understandable in view of the complexity of this preparation. In these experiments, not only were there many fibres in the area studied, but these fibres being both longitudinal and circular were at right angles to each other. Nevertheless the same essential features are discernible, namely, rapid, initial potentials succeeded by slow waves consisting of a base line shift with superimposed intermediate slow activity. The potential II described by Lambert and Rosenblueth cannot be demarcated in these studies. Indeed potential I, as has been indicated, is complex. This complexity of faster frequencies with a spread into the sphere of the slower

activity is understandable in view of the subsequent activation of adjacent muscle fibres.

In the resting phases, that is, when mechanical evidence of contraction is lacking, there is no indication of electrical activity. This is in contradistinction to Bozler's observations in the guinea pig.

It has been noted that the electric potentials preceded the mechanical evidence of contraction. While this is not surprising, an interval of time of this magnitude (1.5–2 sec.) had not been anticipated. The explanation for this time lag probably includes the size of the balloon, the placement of the electrodes, and the inertia of the mechanical system.

The precedence of electrical activity would indicate that what has been recorded is not artefact. Moreover, movement of the electrodes over the small intestinal surface, in the ileostomy experiment, produced activity clearly discernible from the activity shown. The activity produced by such artefact
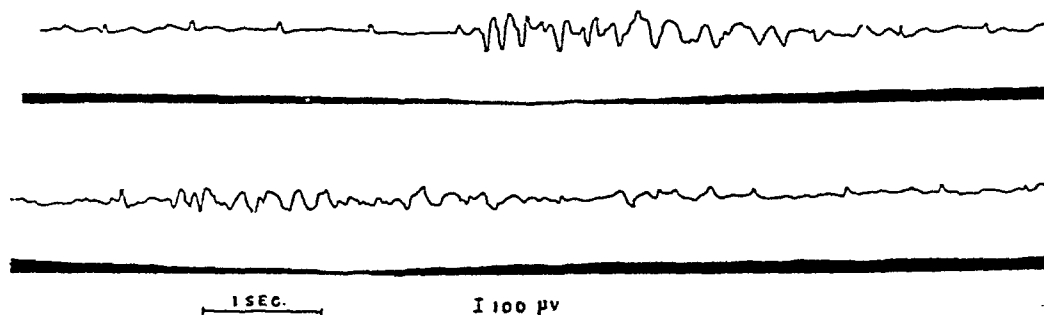
Fig. 2. Continuous record by intubation technique through two relatively short contractions. Upper tracings are electric potentials of small intestine and the E. K. G. Lower tracings are from the tambour and record mechanical evidence of contraction. Record shows onset of electrical activity before the mechanical evidence. The three phases of the electrical activity are evident.

consisted of slow waves to the extent of amplification with interspersed spikes resembling electrode "pops". In intubation experiments, artefacts were encountered only during a violent contraction often associated with cramping and borborygmus, and in these instances the tube was later found by fluoroscopy to have shifted position. However, even in these instances the artefacts were preceded by distinct activity such as shown in the illustrations.

CONCLUSIONS

1. Electric potentials can be obtained from the intact human small intestine or the small intestine previously exteriorised.

2. These electric potentials correlate with the mechanical evidence of contraction.

3. In essential respects these electric potentials are similar to the activity of isolated preparations of smooth muscle.

4. The similarity consists of fast initial spikes followed by long base line shifts with superimposed intermediate activity.

REFERENCES

(1) ROSENBLUETH, A. AND D. McK. RIOCH. This Journal **103**: 681, 1933.
(2) LAMBERT, E. F. AND A. ROSENBLUETH. This Journal **114**: 147, 1935.
(3) ROSENBLUETH, A., H. DAVIS AND B. REMPEL. This Journal **116**: 387, 1936.
(4) BERKSON, J. This Journal **104**: 62, 1933.
(5) BOZLER, E. This Journal **124**: 502, 1938.
(6) BOZLER, E. This Journal **127**: 301, 1939.
(7) MILLER, T. G. AND W. O. ABBOTT. Am. J. Med. Sci. **187**: 595, 1934.

PLASMA RETENTION, URINARY EXCRETION AND EFFECT UPON CIRCULATING TOTAL RED CELL VOLUME OF INTRAVENOUS GELATIN IN NORMAL DOGS[1]

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In a previous paper (1) it was shown that intestinal capillaries which were injured sufficiently to permit the partial or complete passage of serum proteins through their walls allow the passage of only 35 to 60 per cent of plasma gelatin. In view of this fact the rate of disappearance of gelatin from plasma is of interest especially with reference to the use of gelatin solutions as blood substitutes. Amberson (2) cites data reported by various authors which show that infused gelatin had completely disappeared from the blood in from 2 to 40 hours after its infusion in normal animals. There was considerable difference of opinion regarding the rate of its disappearance, probably because indirect methods for determining its concentration in the blood were used.

The purpose of the investigations reported here was the study of: *a*, the disappearance of intravenously injected gelatin-saline solutions from the blood after single and repeated injections in normal dogs, and the excretion of gelatin in the urine; *b*, the effect of intravenously injected gelatin upon the total red-cell volume.

PROCEDURE AND METHODS. *Disappearance from the blood and the excretion of gelatin.* Unanesthetized dogs were used. A plasma volume determination was done using the method of Gibson and Evelyn (3) and blood was drawn for analysis. The bladder was catheterized and emptied. The animals were then given an intravenous injection of gelatin-saline solution (4 to 7 per cent) equal to 1 per cent of the body weight. In the case of 4 dogs blood was drawn for analysis and plasma volume determinations were made at intervals of $\frac{1}{2}$, 6, 24, 48 and 72 hours. All urine was collected for analysis at intervals of 6, 24, 48 and 72 hours, the period of collection being terminated always by catheterization. Four other dogs received gelatin-saline injections at intervals of 24 hours for 4 days. Blood was drawn for analysis and plasma volume determinations were done just prior to these injections. All urine was collected for analysis at intervals of 24 hours and each period of collection was again terminated by catheterization.

The serum analyses were done according to a previously published procedure (1). It was necessary to devise a procedure for the determination of gelatin in urine. It was found that 5 per cent trichloracetic acid will not precipitate gelatin from urine, but it will precipitate other proteins which may be present. Gelatin is precipitated by tungstic acid. From 0.2 to 6.0 ml. of urine are pipet-

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ted into a 60 ml. centrifuge tube used in the Sorvall angle centrifuge, the volume of urine to be used being estimated by a preliminary precipitation. The total volume is brought to 9.0 ml. by the addition of distilled water; 0.5 ml. of 10 per cent sodium tungstate and 0.7 ml. of $\frac{2}{3}$ N sulphuric acid are added. In some cases it is necessary to add up to a total of 1.0 ml. of sulphuric acid before precipitation is complete. The tubes are allowed to stand for 15 or 20 minutes if flocculation does not occur immediately and are then centrifuged for 15 minutes or until the supernatant fluid is clear. The completeness of precipitation is checked by adding 1 drop of $\frac{2}{3}$ N sulphuric acid to the clear supernatant fluid. If more precipitate forms, several drops of the acid are added, and the tube is centrifuged again. The fluid is then decanted and the precipitate is washed with 4 ml. of a solution made by mixing 1 part of 10 per cent sodium tungstate, 1 part of $\frac{2}{3}$ N sulphuric acid and 18 parts of distilled water. The tube is centrifuged and decanted, and this washing process is again repeated. Decantation causes no appreciable loss of precipitate, which was ascertained by using an asbestos plug filter in some cases.

The nitrogen in the precipitate is determined according to the method of Van Slyke (4) and Van Slyke and Kugel (5). After digestion and neutralization the solution is transferred with washing to a 25 milliliter volumetric flask and brought up to volume with distilled water. Ten ml. aliquots of the solution are used for the manometric determination of nitrogen, but in some cases it is necessary to use greater or smaller aliquots depending upon the quantity of nitrogen present. The nitrogen values are converted to gelatin values by the usual factor 6.25.

All urine samples were tested for the presence of proteins other than gelatin by precipitation in a 5 per cent trichloracetic acid solution. In no case was protein other than gelatin found.

All determinations were done in duplicate, and those not checking within 5 per cent were repeated.

The effects of gelatin on the total red cell volume. Observations on the total red cell volume were made using the method of Gibson and Evelyn (3). Unanesthetized dogs were used. All blood samples were drawn from the jugular or femoral vein without stasis. Control determinations of total red cell volumes were made after which the animal was given intravenously a quantity of gelatin solution equal to 1 per cent of the body weight. Some animals received a single injection of 6 to 7 per cent gelatin after which the red cell volume determinations were made at intervals of $\frac{1}{2}$, 6, 24, 48 and 72 hours. Other animals received approximately 4 per cent gelatin solutions injected at 24 hour intervals for 4 days, the total red cell volume being determined prior to each injection. In some of these animals injections of 6 per cent gelatin solutions were continued for 7 or 8 days after which the total red cell volume was again determined.

RESULTS AND DISCUSSION. In order to test the accuracy of the urinary gelatin determinations, a known quantity of gelatin was added to urine which had been collected during some of the preliminary experiments and was known to contain gelatin. The results are given in table 1. It will be seen that the maximum variations from the theoretical concentrations is less than 7 per cent.

Disappearance from the blood and excretion of gelatin. Complete data obtained

following a single injection of a gelatin solution in a typical experiment are given in table 2, and the data on the disappearance of gelatin from the plasma and its excretion in the 4 animals in this group are summarized in table 4.

Approximately 50 per cent of the injected gelatin had disappeared from the plasma within 30 minutes after injection, and by the end of 72 hours only traces of gelatin remained in the plasma. This means that gelatin began leaving the blood soon after its injection and left rather rapidly. However, only 23 to 35

TABLE 1

Recovery of gelatin added to urine

TOTAL GELATIN CONCENTRATION	DETERMINED ORIGINAL GELATIN CONCENTRATION	CONCENTRATION OF ADDED GELATIN	
		Found	Theoretical
gram per cent	*gram per cent*	*gram per cent*	*gram per cent*
0.67	0.15	0.52	0.55
0.67	0.15	0.52	0.55
0.83	0.27	0.56	0.55
0.84	0.27	0.57	0.55
1.61	0.80	0.81	0.87
1.63	0.80	0.83	0.87

TABLE 2

Disappearance of gelatin from blood following a single intravenous injection and its excretion in urine. Dog 3. Received 10.04 grams gelatin as a 7 per cent solution

HOURS AFTER GELATIN INJECTION	PLASMA VOLUME	SERUM PROTEIN CONC.	SERUM GELATIN CONC.	SERUM N.P.N.	TOTAL SERUM PROTEIN	TOTAL SERUM GELATIN	GELATIN LOST FROM PLASMA	HOURS AFTER GELATIN INJECTION	URINE VOLUME	URINE GELATIN CONC.	TOTAL URINE GELATIN
	ml.	*gram per cent*	*gram per cent*	*mgm. per cent*	*gram*	*gram*	*gram**		*ml.*	*gram per cent*	*gram**
Control	600	6.21		33.5	37.26						
½	773	4.97	0.71	35.9	38.42	5.49	4.55	0–6	193	0.29	0.56
6	678	5.22	0.43	35.7	35.39	2.92	7.12				
24	678	5.38	0.23	37.7	36.48	1.56	8.48	6–24	217	0.70	2.08
48	657	5.72	0.16	23.3	37.58	1.05	8.99	24–48	175	0.22	2.47
72	760	5.91	0	26.3	44.92		10.04	48–72	297	0.11	2.80

* Cumulative values.

per cent of that which left the plasma was excreted in the urine. Whether the gelatin not accounted for in the urine is stored or metabolized is not known. Brunschwig et al. (6) have obtained evidence which suggests that at least a portion of intravenously injected gelatin is metabolized.

The excretory rate of gelatin diminished as the serum concentration decreased, as one would expect. The serum gelatin concentrations given in table 2 are quite representative of those in the other dogs of this group.

The gelatin induced a diuresis during the first 6 hours following its injection. During this period the hourly urinary volume is about 100 per cent greater than subsequent hourly excretion rates. This confirms unpublished evidence ob-

tained on anesthetized dogs in which it was found that the urinary volume was from 2 to 18 times greater per unit of time after gelatin than during the control period.

TABLE 3

Disappearance of gelatin from blood during repeated injections and its excretion in urine. Dog 7. Received 4 per cent gelatin solution

DAYS	GELATIN INJECTED	PLASMA VOLUME	SERUM PROTEIN CONC.	SERUM GELATIN CONC.	SERUM N.P.N.	TOTAL SERUM PROTEIN	TOTAL SERUM GELATIN	GELATIN LOST FROM PLASMA	URINE VOLUME	URINE GELATIN CONC.	TOTAL URINE GELATIN
	*gram**	*ml.*	*gram per cent*	*gram per cent*	*mgm. per cent*	*gram*	*gram**	*gram**	*ml.*	*gram per cent*	*gram**
Control		800	7.05		24.7	56.4					
1†	8.86	891	6.52	0.18	35.9	58.1	1.60	7.26	880	0.12	1.06
2	17.72	874	6.30	0.37	24.9	55.1	3.23	14.49	525	0.53	3.84
3	26.39	874	6.05	0.47	22.0	52.9	4.11	22.28	700	0.40	6.64
4	35.06	908	5.99	0.32	45.5	54.4	2.91	32.15	405	0.66	9.31

* Cumulative values.
† All samples were drawn at the end of a 24 hour period.

TABLE 4

Summary of data on gelatin disappearance from the blood and its excretion in the urine

DOG	SINGLE INJECTION OF 6 TO 7 PER CENT GELATIN SOLUTION					DOG	REPEATED INJECTIONS OF 4 PER CENT GELATIN SOLUTION			
	Hours after injection						Days			
	½	6	24	48	72		1	2	3	4
1 A*...		54.7	81.3		95.5	5 A....	85.3	78.4	91.0	93.7
B....		19.7	21.7		26.9	B....	31.6	31.9	30.5	33.0
2 A....	55.7	71.1	86.7	96.7	98.8	6 A....	91.9	81.1	90.6	85.3
B....		17.0	28.8	33.3	35.0	B....	25.7	33.0	31.5	33.3
3 A....	45.3	70.9	84.5	89.5	100	7 A....	81.9	81.8	84.4	91.7
B....		7.9	24.5	27.5	27.9	B....	14.6	26.5	29.8	29.0
4 A....	47.6	64.7	84.3	93.0	98.2	8 A....	80.9	82.1	86.3	85.8
B....		17.1	20.3	22.2	22.7	B....	24.4	32.1	32.6	35.1

* A: Per cent of total injected gelatin lost from plasma. B: Per cent of total gelatin lost from plasma found in urine.

In one dog (table 2), the plasma volume increased and remained elevated for 3 days. In all of the animals the plasma volume was somewhat elevated 30 minutes after the injection of gelatin, but in 3 dogs it had returned to approximately the control level in 6 hours.

Data obtained with repeated gelatin injections in a typical experiment are given in table 3 and a summary of data is given in table 4.

There is some increase in the serum gelatin concentration and in the total serum gelatin with repeated injections, although the per cent of the injected gelatin which left the plasma is of the same order as that found with a single injection. The fraction of the gelatin leaving the plasma and found in the urine was only slightly greater than with a single injection. In the normal dog there is little tendency for the gelatin to accumulate in the blood. When given at 24 hour intervals the dog is able to store or metabolize gelatin with the same degree of efficiency as after a single injection.

In contrast with the single injection experiments there was a consistent increase in the plasma volume with repeated injections. On the average this was

TABLE 5

The effect of a single injection of gelatin solutions on the total red cell volume

DOG	CONCEN-TRATION OF GELATIN SOLUTION INJECTED		HOURS AFTER GELATIN INJECTION					
			Con-trol	½	6	24	48	72
	gram per cent							
1	6.30	Hematocrit reading	44.6		38.3		38.3	35.2
		Red cell volume (ml.)............	611		458		451	395
		Per cent change from control value in R.C. volume			−25		−26	−35
2	6.84	Hematocrit reading	46.0	34.8	37.0	36.6	35.6	31.2
		Red cell volume (ml.)	273	186	197	179	189	144
		Per cent change from control value in R.C. volume		−32	−28	−34	−31	−47
3	7.17	Hematocrit reading	50.8	35.8	36.4	37.3	37.8	36.3
		Red cell volume (ml.)	620	431	388	403	399	433
		Per cent change from control value in R.C. volume		−31	−37	−35	−36	−30

a 10 per cent increase and it was maintained throughout the experimental period. There was a considerable decrease in the serum protein concentration, but the changes in the total serum protein were not significant. This can be explained by the increase in the plasma volume.

The observations reported here show that in the normal dog intravenously administered gelatin leaves the plasma at a rather rapid rate. This raises the question as to whether gelatin solutions would be very effective as a blood substitute. Although the evidence is not very extensive, it has been shown in hypoproteinemic dogs and humans (7, 8) that the transfused plasma and serum proteins leave the blood stream very rapidly. It is possible that in shock the infused gelatin will not leave the blood stream as rapidly as in the case of normal dogs. In the case of 3 dogs in shock which received gelatin we found (unpub-

lished data) that after 21 hours the gelatin concentration was 48 per cent the concentration immediately after injection, after 19 hours 34 per cent and after 24 hours 84 per cent.   In the series reported here the average gelatin concentration 24 hours after injection is approximately 30 per cent that found ½ hour after injection.

The effect of gelatin upon the total red cell volume.   The effect of single injections of gelatin solutions (6.3 to 7.17 per cent) on the total red cell volume will be found

TABLE 6

The effect of repeated injections of gelatin solutions on the total red cell volume

DOG	DAYS	CONCEN-TRATION OF GELA-TIN SOLU-TION INJECTED	HEMA-TOCRIT READ-ING	TOTAL RED CELL VOLUME	CHANGE FROM CONTROL VALUE IN R.C. VOLUME	DOG	DAYS	CONCEN-TRATION OF GELA-TIN SOLU-TION INJECTED	HEMA-TOCRIT READ-ING	TOTAL RED CELL VOLUME	CHANGE FROM CONTROL VALUE IN R.C. VOLUME
		gram per cent		*ml.*	*per cent*			*gram per cent*		*ml.*	*per cent*
1	Control	3.81	53.0	986		4	Control	3.92	49.8	717	
	1	3.87	52.5	900	−9		1	3.92	40.8	560	−22
	2	3.87	47.5	837	−15		2	3.92	37.7	476	−34
	3	3.87	45.1	774	−22		3	3.92	36.8	458	−36
	4		45.7	736	−25		4	6†	34.4	427	−41
							4–11	6†	30.3	365	−49*
2	Control	4.35	48.3	586		5	Control	4.12	48.5	744	
	1	4.35	44.7	658	+12		1	4.12	41.5	632	−15
	2	4.23	39.3	527	−10		2	4.03	42.5	646	−13
	3	4.23	38.5	492	−16		3	4.03	40.7	600	−19
	4		37.8	486	−17		4	6†	36.9	531	−29
							4–12	6†	35.0	396	−47*
3	Control	4.35	44.2	680		6	Control	4.12	52.4	929	
	1	4.35	41.1	711	+5		1	4.12	50.5	945	+2
	2	4.23	40.0	583	−14		2	4.03	51.1	966	+4
	3	4.23	37.3	560	−18		3	4.03	44.8	811	−13
	4		36.4	539	−21		4	6†	41,5	668	−28
							4–12	6†	38.7	573	−38*

* Determinations of the total red cell volume done 24 hours after the last injection.
† Approximately 6 per cent by weight allowing for the estimated water content.

recorded in table 5.   There was an almost immediate decrease in the total red cell volume which remained practically unchanged for 48 hours.   In two cases there was a further decrease after 72 hours.

The effect of repeated gelatin injections will be found in table 6.   In these experiments the concentration of the injected gelatin was approximately 4 per cent in contrast with the above single injections.   These injections were given daily for 4 days.   In three animals there was a slight decrease at the end of 24 hours while in the others there was a slight increase in the total red cell volume.

In all animals, except no. 6, the decrease had become evident by the end of the second day. In most instances as the injections proceeded the diminution in total red cell volume was not directly proportional to the amount of gelatin injected. In three animals (dogs 4, 5 and 6) the injections were continued for 7 or 8 days beyond the usual 4 day period but with approximately 6 per cent gelatin instead of 4 per cent gelatin. At the end of this period there was a further decrease in the total red cell volume. However, this decrease was less than one might expect from the results with single injections of 6 per cent gelatin as will be seen by comparing the data in table 5 with those on dogs 4, 5 and 6 in table 6.

It is quite evident that the gelatin which was used in these experiments causes a decrease in the circulating total red cell volume. When the animals received repeated injections of 4 per cent gelatin the decrease was not as marked at first as with 6 to 7 per cent solutions. After 12 daily injections of gelatin the animals were not critically anemic. However, this effect must be kept in mind in interpreting results obtained by transfusion with gelatin solutions after hemorrhage, since the mortality found may be due to the anemia rather than to an inadequate maintenance of blood volume.

The cause for the decrease in total red cell volume following injection of gelatin solutions is not known. It is possible that the decrease may be due to hemolysis, or to increased phagocytosis of the red cells by the reticulo-endothelial system. Another possibility is that the size of individual erythrocytes is decreased. In three animals there was no significant difference between the decrease in the erythrocyte count and the hematocrit reading. If the size of the individual erythrocyte were decreased one would expect to find a greater decrease in the hematocrit reading than in the erythrocyte count.

SUMMARY

1. A procedure for the determination of gelatin in urine has been described.

2. Following a single injection of a gelatin-saline solution approximately 50 per cent of the gelatin left the plasma within 30 minutes. Only traces of gelatin were present after 72 hours.

3. Following a single injection of a gelatin-saline solution from 23 to 35 per cent of the gelatin which left the plasma was found in the urine.

4. With repeated injections of gelatin-saline solutions there was some increase in the serum gelatin concentration and total amount of serum gelatin. The total amount of serum proteins was unaffected, but the serum protein concentration decreased.

5. Following a single injection of 6 to 7 per cent gelatin solutions there is a marked and almost immediate decrease in the circulating total red cell volume which persists for at least 3 days.

6. During repeated injections of 4 per cent gelatin the immediate decrease in red cell volume did not always occur, and when it did occur it was not as marked as with 6 per cent gelatin solutions. With repeated injections there is progressively less effect on the total red cell volume.

REFERENCES

(1) LITTLE, J. M. AND H. S. WELLS.   This Journal **138**: 495, 1943.

(2) AMBERSON, W. R.   Biol. Rev. **12**: 48, 1937.

(3) GIBSON, J. G. AND K. A. EVELYN.   J. Clin. Investigation **17**: 153, 1938.

(4) VAN SLYKE, D. D.   J. Biol. Chem. **71**: 235, 1927.

(5) VAN SLYKE, D. D. AND V. H. KUGEL.   J. Biol. Chem. **102**: 489, 1933.

(6) BRUNSCHWIG, A., V. B. SCOTT, N. CORBIN AND R. MOE.   Proc. Soc. Exper. Biol. and
    Med. **52**: 46, 1943.

(7) SHEARBURN, E. W.   Surg., Gynec. and Obstet. **74**: 343, 1942.

(8) HAYWARD, G. W. AND A. JORDON.   Brit. Med. J. **1**: 462, 1942.

OLFACTORY DISCRIMINATION AFTER DESTRUCTION OF THE ANTERIOR THALAMIC NUCLEI

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The survey of forebrain structures by Swann (1935) and by Ghiselli and Brown (1938) has failed to reveal any structure essential for the olfactory discrimination between oil of anise and sheep dip (creosote). Allen (1941) has criticized this work on the ground that discrimination of these odors might be mediated alternatively by olfactory or trigeminal stimulation, but it should be emphasized that Swann was unable to obtain a discriminative reaction from any animal in which the olfactory bulbs had been destroyed. It seems certain that, in the concentrations used in his experiments, there was no differential trigeminal stimulation.

In a careful series of studies Allen (1941 and earlier) has reported effects upon olfactory reactions only after destruction of the frontal lobes or of the pyriform-amygdaloid complex in dogs. He concluded in summing up his work (1941) that disturbance of the relations between the pyriform-amygdaloid complex and the isocortex results in a loss of discriminative reactions and that the conditioned reaction to a single odor (presence vs. absence of odor) is probably mediated by impulses which pass to the cortex by way of the anterior thalamic nuclei (the principal path not explored in his experiments). It is not clear from his data that he obtained a true olfactory defect. On the contrary, the similarity of symptoms after frontal lobe and pyriform lesions and the indications of occasional discrimination by animals with such lesions suggest that he was dealing with a more general deterioration involving disturbances of the process of comparison, such as have been described after frontal and temporal lesions in monkeys.

Of cerebral systems intimately associated with primary olfactory centers, only the anterior nuclei have not been systematically investigated. Their cortical field is very extensive, including probably all of the infraradial and retrosplenial areas (Lashley, 1941) and the course of the projection fibers is such that the entire field has probably never been isolated from the thalamus in any experimental animal or clinical case. The present experiments were designed to test the effects of complete interruption of the radiations of the anterior nuclei upon a previously formed olfactory discriminative habit.

CORTICAL FIELD OF THE ANTERIOR NUCLEI. Three divisions of the anterior nucleus are distinguishable in the rat; the anterodorsal, anteroventral and anteromedial. The course and termination of their radiations has been described by Lashley (1941) on the basis of retrograde degeneration. The radiation of the anterodorsal nucleus passes forward around the fornix and through the callosal

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fibers just in front of the thalamus to terminate in the Area retrosplenialis granularis. That of the anteroventral follows the same course, spreading more laterad, and terminates in the Area retrosplenialis agranularis. The radiation of the anteromedial nucleus passes forward along the mesial surface of the striatum to the Area infraradiata. The apparent course and termination of the fibers, as determined from lesions producing retrograde degeneration, are shown in figure 1. Section of the radiation results in complete disintegration of all neurons in the nucleus.

METHODS. *Animals.* Black female rats about five months old from a strain derived from a cross of albino with wild gray were used for the experiments.

Training. The apparatus used for training consisted of three small blocks of wood, each containing a well, 30 mm. in diameter and 40 mm. deep, provided
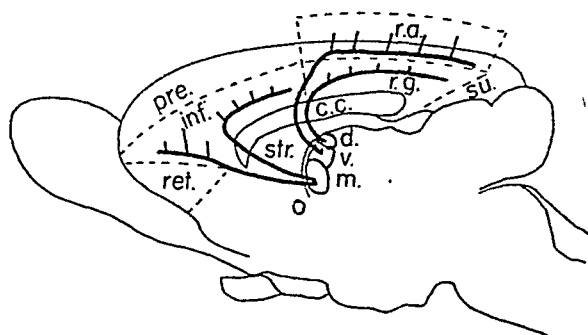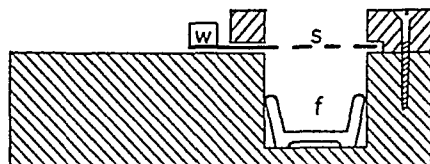
Fig. 1   Fig. 2

Fig. 1. Diagram of the anatomic projection of the anterior nuclei upon the cerebral cortex. *d.*, anterodorsal nucleus; *v.*, anteroventral nucleus; *m.*, anteromedial nucleus; *c.c.*, corpus callosum; *inf.*, area infraradiata; *pre.*, regio praecentralis; *r.a.*, area retrosplenialis agranularis; *ret.*, regio retrobulbaris; *r.g.*, area retrosplenialis granularis; *str.*, corpus striatum; *su.*, area presubicularis. (Terminology of Rose, 1929.)

Fig. 2. Sectional sketch of one of the three training boxes. The circular well is closed by the perforated sliding cover, *s*, weighted by a small lead block, *w*. Food or cotton saturated with an odorous substance is placed in the glass dish, *f*, in the well. The rat opens the cover by clawing at the perforations above the well.

with a glass container at the bottom and with a perforated sliding metal cover which the rats could pull open (fig. 2). The blocks were impregnated and coated with paraffin so that they could be washed and recoated to remove odors. One block was placed in each of the three corners of a square restraining cage, 65 by 65 cm., and the rat admitted at the fourth. Milk-soaked bread was placed in one well and cotton with a few drops of oil of wintergreen in the others. The relative position of the blocks was varied in chance order in successive trials. The rats were given 5 to 10 trials per day until they chose the block containing bread and milk correctly in 28 or more of 30 consecutive trials (criterion of discrimination). Any attempt to open the cover of a well which did not contain food was counted as an error.

Controls. Reaction to position was controlled by chance arrangement of the relative positions in which the three blocks were placed. Non-olfactory cues

from the blocks were eliminated by regular washing and placing the food in each of the three in succession.

Oil of wintergreen is listed by Allen as a trigeminal irritant. Two controls were introduced to determine whether the rats were reacting to such stimulation and merely avoiding the wintergreen. *a.* Milk-soaked bread was added to the wells containing wintergreen after errorless performance had been established in operated animals. This led in every case to a confusion, with chance scores, showing that the animals had been reacting to the food odor and that they could detect the food odor when mixed with wintergreen. *b.* When discrimination followed the cerebral operations, the animals were subjected to a second operation involving destruction of the olfactory bulbs. Total removal of the bulbs permanently abolished the discrimination, showing that the rats were not reacting to the trigeminal component of the wintergreen stimulation.

Operation. The animals were anesthetized with ether, the skull trephined near the midline on each side. A thermocautery was thrust diagonally downward and forward through the region of the genu to a sufficient depth to pass through the striatum, to sever the radiation of the anteromedial nucleus. A shallow transverse incision to the depth of the corpus callosum was also made above the septum to interrupt the radiations of the anterodorsal and anteroventral nuclei.

Analysis of lesions. At the termination of the experiment the brains were fixed in alcohol, sectioned and stained with thionin. With two exceptions, the animals had survived long enough to permit of total degeneration of injured cells, so that the boundaries of degenerated areas were clearly defined. Sections through the thalamus were examined for retrograde degeneration and camera lucida sketches made to show the approximate amount of degeneration in each nucleus.

EXPERIMENTAL RESULTS. Eight animals were carried through training, operation and postoperative tests. All gave evidence of retention of the discriminative reaction after operation. In five of the eight destruction of the radiation was incomplete so only the records of the three cases which are crucial for the problem will be reported.

No. 1. This animal made 37 correct choices in 38 trials after 15 trials of training. She was then rested ten days and given preoperative retention tests, making no error. Operated and retested after 10 days, she gave 50 consecutive errorless trials in postoperative retention tests. The olfactory bulbs were then destroyed. Subsequent tests gave chance scores with no evidence of discrimination.

Sections showed total degeneration of the right anterodorsal, anteromedial and anteroventral nuclei. About 20 normal cells per section (less than 6 per cent of the normal number) were found in the left anterodorsal nucleus. The left anteroventral and anteromedial nuclei were totally degenerated. The nucleus reuniens was totally degenerated on both sides and the right and left dorsomedial nuclei showed some degeneration. The anterior half of the septum was destroyed and the third ventricle much distended.

No. 2. Thirty-seven of 38 trials were correct after 15 trials of training. Preoperative retention tests gave 10 consecutive errorless trials. She did not attempt to open the slides until the fourteenth day after operation, then gave 29 of 30 trials without error. The second operation, to destroy the olfactory bulbs, failed to remove the greater part of the left. Ensuing tests gave errorless performance for 3 days. The rat then developed an infection of the remaining bulb and the score fell to chance.

Sections showed total degeneration of the anterodorsal, anteroventral and anteromedial nuclei and of the nucleus reuniens on both sides. There was also extensive degeneration in the median division of the nucleus ventralis. The dorsal margin of the septum was destroyed.

No. 3. After 22 trials of training 40 errorless choices were made in 42 trials. The operation followed without the usual preoperative rest period. Following operation the animal had to be force-fed for several days and did not attempt to open the boxes until the twentieth day after operation. She then gave 39 correct responses in 42 trials. The olfactory bulbs were next destroyed. Subsequent training gave only 4 errorless in 40 trials, with no evidence of improvement.

Sections showed the anterodorsal, anteroventral, anteromedial and reuniens nuclei totally degenerated on both sides. The dorsal part of the medial division of the ventral nucleus and the mesial portion of the lateral nucleus also showed bilateral degeneration. The septum was entirely destroyed and there was slight invasion of the dorsal convexity of both hippocampal lobes.

These three cases give crucial evidence of the persistence of olfactory discrimination after interruption of the cortical radiations of the anterior nuclei. In numbers 2 and 3 all neurons of the anterior group had disappeared and in number 1 there were only a few possibly functional cells in the left anterodorsal nucleus. All of the animals discriminated accurately after the cerebral operation. Numbers 1 and 3 lost the habit after destruction of the olfactory bulbs and number 3 failed to reacquire it within twice the amount of training required for original learning. In number 2 the discrimination persisted after partial destruction of the olfactory bulbs but disappeared with infection of the remaining olfactory tissue. Thus it is clear that the discriminative reaction was mediated by the olfactory bulbs and that it was not abolished by total degeneration of all neurons in the three divisions of the anterior nucleus.

In the remaining five animals there was less extensive degeneration in the anterior nuclei, ranging from the persistence of a few normal cells in both anterodorsal nuclei to the preservation intact of one anteroventral nucleus. Their behavior was completely consistent with that of the three reported above.

DISCUSSION. By analogy with other senses one would expect to find an olfactory area in the isocortex essential for the most highly specialized discriminative reactions, with more primitive sensorimotor reactions mediated by thalamic or other subcortical mechanisms. The anatomic connections of the anterior thalamic nuclei have led Herrick (1931) and Kappers, Huber and Crosby (1936) to ascribe to these structures the correlation of olfactory with general somatic reactions. The cortical field of the anterior nuclei is extensive; equal

in area to the visual, auditory, or somesthetic fields, and has as yet no demonstrated function. There is therefore good reason to anticipate olfactory loss after total elimination of this system. The present experiments, however, have not revealed any disturbance of olfactory function after section of the radiations and total degeneration of the cells of the anterior nuclei. They suggest that the system is not involved in olfactory functions. It should be pointed out, however, that the stimuli used were intense and perhaps simple. The olfactory apparatus of macrosmatic animals seems to be specialized for differentiation of organic odors to which man is relatively insensitive, and experiments with such substances as stimuli must be carried out before final conclusions concerning the olfactory functions of the anterior nuclear system are justified.

SUMMARY

Olfactory discrimination of rats was tested after interruption of the cortical radiations of the anterior group of nuclei. Discrimination between the odors of oil of wintergreen and of bread and milk was not disturbed by total bilateral degeneration of the anterodorsal, anteroventral and anteromedial nuclei, with additional involvement of the septum. Removal of the olfactory bulbs permanently abolished the discrimination, showing that the reaction was based upon olfactory and not trigeminal stimulation.

REFERENCES

ALLEN, W. F. This Journal 132: 81, 1941.
GHISELLI, E. E. AND C. W. BROWN. J. Comp. Psychol. 26: 93, 1938.
HERRICK, C. J. An introduction to neurology. W. B. Saunders, 5th ed., 1931.
KAPPERS, C. W. A., G. C. HUBER AND E. C. CROSBY. The comparative anatomy of the nervous system of vertebrates, including man. 2 vols. Macmillan, 1936.
LASHLEY, K. S. J. Comp. Neurol. 75: 67, 1941.
ROSE, M. J. f. Psychol. u. Neurol. 40: 1, 1929.
SWANN, H. G. This Journal 111: 257, 1935.

THE EFFECTS OF OCCLUSION OF EXPERIMENTAL CHRONIC PATENT DUCTUS ARTERIOSUS ON THE CARDIAC OUTPUT, PULSE AND BLOOD PRESSURE OF DOGS[1]

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The possibility of the surgical repair of patent ductus arteriosus (botalli) in human beings, first successfully demonstrated by Gross and Hubbard (5), has stimulated renewed interest in this condition. When a patent ductus arteriosus is present, the abnormal shunting of blood from the aorta to the pulmonary artery leads to a train of pathologic changes particularly in the heart and the large arteries. It is not the purpose of this paper to discuss arteriovenous aneurysms in detail but there are two important differences between patent ductus arteriosus and peripheral arteriovenous fistula to which attention may be called: 1, the output of the right and that of the left ventricle is unequal in the presence of a shunt between the aorta and pulmonary artery but is equal in peripheral arteriovenous fistula, and 2, in patent ductus arteriosus the increased volume flow is in the pulmonary circulation alone while in arteriovenous fistula an increased volume flow occurs in both the systemic and pulmonary vessels. A condition similar to patent ductus arteriosus in man may be produced experimentally in dogs by lateral anastomosis between the aorta and the left pulmonary artery. It may also be produced by end-to-side anastomosis of the left subclavian artery to the main or left pulmonary artery (2).

The purpose of the present experiments was to determine the immediate effect of occlusion of a long standing experimental patent ductus arteriosus on the blood pressure, pulse and cardiac output of the right ventricle and of the left ventricle in dogs. Additional experiments dealt with the immediate effect on the cardiac output of the right ventricle only of an experimental ductus.

METHOD. Large dogs fed on kennel ration supplemented with meat were used in all instances. Food was withheld before any procedure was performed. The operations were performed under sodium pentobarbital anesthesia administered intravenously with air given intratracheally under positive pressure. The fourth or fifth left intercostal space was opened. The vessels were occluded by rubber-shod clamps and a side-to-side anastomosis was made between the aorta and the left pulmonary artery. In a similar manner an end-to-side anastomosis was made between the left subclavian and the left pulmonary artery after ligation distally of the left subclavian artery.

Preliminary determination of the cardiac output was done without anesthesia one or more days before the operation in most of the cases. A grain of morphine was administered and the consumption of oxygen was determined by means of the Benedict-Roth spirometer. Samples of blood were obtained under oil

from the right ventricle and from the left ventricle or femoral artery. Potassium oxalate was the anticoagulant. The oxygen consumption was determined before each operation with the animals under anesthesia and again after the operation was completed and the chest closed. While the chest was open, specimens of blood were obtained from the aorta, from the pulmonary artery at various sites, and sometimes from the right ventricle. The oxygen content of the blood was determined manometrically in duplicate on samples of 2 cc. each using the Van Slyke-Niell apparatus (10). The output of the right ventricle[2] was calculated by the Fick principle (3). In each instance where it was possible the output of the left ventricle was also calculated using the oxygen content of mixed aortic and pulmonary artery blood taken from the pulmonary artery in the Fick formula.

The blood pressure was obtained by inserting into an artery a needle connected. with a mercury manometer. This gives a determination near the mean blood pressure.

RESULTS. Eight experiments were performed on seven dogs. In five experiments the animals were maintained for a long period of time and, in three, only the immediate effects of an experimental patent ductus arteriosus were observed.

Following the establishment of a fistula between the aorta and pulmonary artery a turbulent stream of blood from the aorta flowed into the pulmonary artery creating a loud murmur and a thrill so pronounced that it vibrated strongly against the palpating finger. The pulse increased in rate and became collapsing in type, resembling the Corrigan pulse of aortic insufficiency. A capillary pulse was observed in the extremities and a pistol shot sound could be heard over the femoral arteries. The murmur was loud, continuous and machinery-like, with systolic accentuation, best heard posteriorly in the second intercostal space, with the animal lying in a supine position.

In five experiments a fistula between the aorta and pulmonary artery was maintained for 48, 49, 90, 903 and 1109 days respectively. All but one of the animals (no. 2) had an arterio-arterial opening established by side-to-side anastomosis between the aorta and the left pulmonary artery. In no. 2 an end-to-side anastomosis between the left subclavian and the left pulmonary artery was performed. One animal (no. 4) killed three years after operation had the largest persistent opening into the pulmonary artery, measuring 4 by 5.5 mm., as well

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[2] When the cardiac output is determined by the Fick principle in normal dogs the outputs of the right and left ventricles are equal and the determination is considered to be that of either ventricle. When a ductus arteriosus is present, however, the output of the left ventricle is necessarily greater than that of the right ventricle since the left ventricle must expel not only the peripheral blood flow (which returns to the right auricle and ventricle and constitutes the right ventricle output) but also that portion of the left ventricle output which passes through the ductus into the pulmonary circulation and returns to the left auricle and ventricle to be expelled again. When the cardiac output in subjects with a patent ductus is determined in the usual manner by the Fick principle using mixed blood from the right ventricle and arterial blood the value obtained is the output of the right ventricle. To determine the cardiac output of the left ventricle in these subjects by the Fick principle, mixed blood must be obtained from the pulmonary artery.

TABLE 1

Effects of occlusion of chronic experimental ductus arteriosus

DOG NO.	DAYS DUCTUS PRESENT	BODY WT.	PROCEDURE	SOURCE OF BLOOD SAMPLES	OXYGEN CONTENT	A.V. OXYGEN DIFFERENCE	CARDIAC OUTPUT Left and right ventricle equal	Left ventricle output	Right ventricle output	PER CENT OF C.O. FLOWING THROUGH DUCTUS	PER CENT REDUCTION IN C.O. AFTER OCCLUSION OF DUCTUS	OXYGEN CONSUMPTION
		kgm.			*vol.*%	*vol.*%	*cc./min.*	*cc./min.*	*cc./min.*			*cc./min.*
1	35	20.1	Ductus established Cardiac output, no anesthesia				4,475					
	42		Cardiac output, no anesthesia				3,362					
	48	20.5	Chest opened under intravenous anesthesia and blood samples obtained	Aorta Proximal pulmonary artery Distal pulmonary artery Right ventricle	18.68 17.11 18.69 15.32	1.57 3.36		11,911	5,505	46		187
			After ductus occluded for 13 minutes blood samples obtained	Aorta Pulmonary artery Right ventricle	20.47 9.53 10.26	10.94 10.21	1,618 1,832				85 lt.* 66 rt.	
2			Cardiac output, no anesthesia				2,519					
	• 43	18.5	Ductus established Cardiac output, no anesthesia				2,995					
	49	18.5	Chest opened under intravenous anesthesia	Aorta Proximal pulmonary artery Distal pulmonary artery	20.97 15.19 18.72	5.78 2.25		11,700	4,530	39		262
			Ductus occluded 12 minutes	Aorta Pulmonary artery	20.02 12.12	7.90	3,316				72 lt. 27 rt.	
			Ductus occluded 27 minutes	Aorta Pulmonary artery	20.15 11.11	9.04	2,900				75 lt. 36 rt.	
3	15	20.6	Ductus established Cardiac output, no anesthesia				5,719					
	86	21.8	Cardiac output, no anesthesia				4,181					
	90	22.2	Chest opened under anesthesia	Aorta Proximal pulmonary artery Distal pulmonary artery	12.60 9.36 10.99	3.24 1.61		19,570	9,722	50		315
			Ductus occluded 10 minutes	Aorta Pulmonary artery	9.26 3.79	5.47	5,556				72 lt. 54 rt.	
			Ductus reopened	Aorta Pulmonary artery	10.91 8.29	2.62		12,023				

TABLE 1—*Concluded*

DOG NO.	DAYS DUCTUS PRESENT	BODY WT.	PROCEDURE	SOURCE OF BLOOD SAMPLES	OXYGEN CONTENT	A.V. OXYGEN DIFFERENCE	CARDIAC OUTPUT Left and right ventricle equal	CARDIAC OUTPUT Left ventricle output	CARDIAC OUTPUT Right ventricle output	PER CENT OF C.O. FLOWING THROUGH DUCTUS	PER CENT REDUCTION IN C.O. AFTER OCCLUSION OF DUCTUS	OXYGEN CONSUMPTION
		kgm.			vol. %	vol. %	cc./min.	cc./min.	cc./min.			cc./min.
901		19.9	Cardiac output, no anesthesia	Right ventricle Femoral artery	7.75 12.50	4.75	4,400					209
903		19.9	Cardiac output, intravenous anesthesia (breathing O₂)	Right ventricle Femoral artery	9.03 16.20	7.17	3,696					265
			Chest opened under anesthesia	Aorta Proximal pulmonary artery Distal pulmonary artery	14.70 6.13 10.23	8.57 4.47		5,928	3,092	53		
			Ductus occluded for 3 minutes	Pulmonary artery Aorta	4.97 14.91	9.94	2,666				55 lt. 14 rt.	
4	60	16.9 14.9	Ductus established Cardiac output, no anesthesia				1,524					
	119	15.3	Cardiac output, no anesthesia				2,273					
	220	17.55	Cardiac output, no anesthesia				1,475					
	1,190	17.5	Chest opened	Aorta Proximal pulmonary artery Distal pulmonary artery	16.6 11.7 14.5	4.9 2.1		6,619	2,837	43		139
			Ductus occluded 5 minutes	Aorta Pulmonary artery	17.80 4.17	13.63	1,020				85 lt. 64 rt.	
			Ductus reopened	Aorta Pulmonary artery at ductus Distal pulmonary artery	18.9 18.1 18.6							
			Average..................................							46	74 lt. 44 rt.	

* Refers to left and right ventricle output.

as the loudest murmur. The opening in no. 3 was 2 by 1.5 mm. With one exception the dogs remained in excellent condition. Number 3 gradually lost 2.1 kgm. in weight over a period of 14 months and suffered a mild degree of anemia; the hemoglobin was 80 per cent, the red blood cell count 5.44 million and the cell volume was 41 (Wintrobe). None of the dogs developed peripheral edema or symptoms of cardiac failure; the venous pressure was not elevated, and

no great deviation from normal was observed in the mean blood pressure. In these experiments the ductus was occluded from 3 to 27 minutes and measurements of the circulation were made before and after the occlusion (table 1).

Included in table 1 are the calculated values of the cardiac output of the left ventricle. These were calculated by the Fick formula from samples of

TABLE 2

Immediate effects of establishing an experimental ductus

DOG NO.	DATE	BODY WT.	PROCEDURE	SOURCE OF BLOOD SAMPLES	OXYGEN CONTENT	A.V. OXYGEN DIFFERENCE	CARDIAC OUTPUT	PER CENT INCREASE IN OXYGEN CONTENT OF BLOOD SAMPLES FROM AORTA	PER CENT INCREASE OR DECREASE IN C.O. AFTER DUCTUS MADE	OXYGEN CONSUMPTION
		kgm.			*vol. %*	*vol. %*	*cc./ min.*			*cc./ min.*
5	12-1 -39		Cardiac output, no anesthesia				3,836			
	12-4 -39	17.2	Chest opened under anesthesia and blood samples taken	Pulmonary artery	17.88	4.19	3,365			141
				Aorta	22.07					
			After ductus established Ductus 8 mm.	Proximal pulmonary artery	22.70					
				Distal pulmonary artery	23.25					
				Aorta	23.03			4		
				Right ventricle	17.92	5.11	3,875		13 increase	
6	12-11-39	13.0	Chest opened	Pulmonary artery	15.44	7.77	1,686			131
				Aorta	23.21					
			Ductus established Ductus 10 mm.	Proximal pulmonary artery	26.40					
				Distal pulmonary artery	25.93					
				Aorta	24.55			5		
				Right ventricle	15.72	8.83	1,484		12 decrease	
7	12-14-39	15.4	Chest opened	Pulmonary artery	15.10	5.28	2,123			112
				Aorta	20.38					
			Ductus established	Proximal pulmonary artery	15.28	5.49	2,040		4 decrease	
			Ductus 4 mm.	Distal pulmonary artery	14.85	5.92	1,892		11 decrease	
				Aorta	20.77			2		
Average								4	3.6 decrease	

mixed blood of the aorta and the pulmonary artery, obtained from the left pulmonary artery. The cardiac output of the left ventricle was roughly double that of the right ventricle and, in five experiments, the average blood flow through the ductus, expressed as per cent of the output of the left ventricle, was 46. Comparison of the oxygen content in samples of blood from the pul-

monary artery, as shown in tables 1 and 2, demonstrates that blood in the pulmonary artery is more evenly mixed in dogs in which the experimental patent ductus arteriosus has been present for a long time. Samples of blood from the pulmonary artery can be used to calculate the output of the left ventricle with more accuracy in experiments of long duration rather than in acute experiments.

In the second group, consisting of three experiments, determinations were made of the cardiac output of the right ventricle and pulse immediately before and after establishment of an experimental ductus arteriosus. The results are given in table 2. The experimental production of such a fistula is attended immediately by the following changes in the circulation: The pulse increases from 20 to 40 beats per minute. Presumably the output of the left ventricle is

TABLE 3

Effects of occlusion of experimental patent ductus arteriosus of long standing on the pulse rate and the blood pressure in the aorta and pulmonary artery (dog no. 1)

TIME	BLOOD PRESSURE		Pulse
	Aorta	Pulmonary artery	
	mm. Hg	*mm.* Hg	
2:25			130
2:33			120
2:50			110
2:55	72 proximal*	16 proximal	
2:58	68 distal	18 distal	
3:03	88 proximal†		
3:05	80 distal		105
3:08	68 proximal		
3:15			110
3:18	Occlusion of ductus		
3:20	110 proximal	16 proximal	
3:22	112 distal	16 distal	
3:30			70

* Refers to insertion of needle of blood pressure instrument proximal or distal to opening of ductus.

† Blood pressure in femoral artery was 110 mm. Hg at this time.

increased by the flow of blood through the ductus, which returns to the left auricle and ventricle. This value was not determined for the reasons given above. The output of the right ventricle was not altered significantly. The average increase in the oxygen content of the arterial blood in the three experiments was approximately 4 per cent. This increase is at least a partial manifestation, however, of an increased metabolism resulting from the anesthesia and operation.

In four experiments the pulse, and blood pressure in the aorta and pulmonary artery were recorded before and after occluding the experimental patent ductus arteriosus and the results were identical in each. The results of a typical experiment are given in table 3. It is seen from the table that occlusion of an experimental ductus is followed by a sharp rise in the blood pressure in the aorta,

a slowing of the pulse and no essential change in the blood pressure in the pulmonary artery.

The determinations of the consumption of oxygen before and immediately after operation, with the animals under anesthesia, indicate an increase in the consumption of oxygen. For this reason, the average consumption of oxygen was used in the calculations rather than the basal consumption of oxygen. Basal conditions were not approached except when the preliminary determinations of the cardiac output were obtained. The factors of anesthesia and operation served to increase the consumption of oxygen and the cardiac output, but the comparison between the output and other determinations made before and after occluding the patent ductus arteriosus is considered to indicate accurately the effects of such occlusion.

DISCUSSION. The immediate effects of occlusion of a chronic experimental patent ductus arteriosus (tables 1 and 3) are 1, an increase in the systemic arterial blood pressure; 2, a slowing of the pulse, and 3, a decrease in the cardiac output of both ventricles. The blood pressure in the pulmonary artery changes little and remains well below one-third that in the aorta.

The immediate increase in the blood pressure in the aorta, following the closure of the ductus arteriosus necessarily follows diversion of the entire output of the left ventricle into the aorta distal to the occluded ductus.

The cardio-inhibitory reflex mechanisms mediated by the vagus nerve and carotid sinus result in a slowing of the pulse and a reduction in the minute volume output. The output of both ventricles becomes equal when the ductus arteriosus is occluded, and is reduced from 14 to 66 per cent of the original value in the case of the right ventricle and from 55 to 85 per cent of the left ventricular output. The average reduction in the cardiac output of the left ventricle was 74 per cent and that of the right ventricle 44 per cent.

A factor in the slowing of the pulse following occlusion of an experimental ductus arteriosus might be the suddenly decreased output of the left ventricle. Grollman (4), in a discussion of arteriovenous aneurysm, stated, "The bradycardia phenomenon refers to the drop in pulse rate observed on occluding the arteriovenous communication. This drop in pulse rate might be looked upon as a concomitant of a decrease in cardiac output. Lewis and Drury (9) explain it as a result of an increase in vagal tone. In view of the studies [of Harrison, Dock and Holman, (6)] the first mentioned explanation seems more likely."

The decrease in cardiac output after occlusion of the ductus is accompanied by a marked degree of deoxygenation of blood samples taken from the pulmonary artery, compared to the oxygen content of samples of blood from the aorta. The latter changes little as long as the animals receive an adequate supply of oxygen through the intratracheal catheter.

The depression of the cardiac output following occlusion of the ductus arteriosus persists for more than a few minutes as illustrated by the experiment (no. 2) in which samples of blood from the pulmonary artery and the aorta were obtained 12 and 27 minutes after the ductus was occluded by a clamp and the cardiac output determined.

There was no pronounced change in the blood pressure in the pulmonary artery following occlusion of the experimental ductus arteriosus. This is in accordance with the previous findings of Levy and Blalock (8) who connected the left pulmonary with the left subclavian artery in an end-to-end anastomosis. The blood pressure in the pulmonary artery remained at a low level, a result consistent with a low peripheral resistance in the lung.

In dogs with experimental patent ductus arteriosus blood flows from the aorta into the pulmonary artery rather than the reverse. This is demonstrated by the elevated content of oxygen in samples of blood obtained from the pulmonary artery near the ductus arteriosus, compared to the oxygen content of samples of blood from the right ventricle or pulmonary artery near the semilunar valves, when the experimental ductus arteriosus was open (tables 1 and 2). This would be expected in view of the difference in pressures in these vessels indicated in table 3.

The flow of blood through the ductus arteriosus is a considerable fraction of the output of the left ventricle. According to the determinations in man and experimental dogs, made by Eppinger, Burwell and Gross (2) by means of the Fick principle, and by the acetylene method employed by Keys (7) in man, the volume flow of blood passing through the ductus arteriosus amounts to one-third of the output of the left ventricle in average cases and to one-half in severe cases. The determinations of the flow through the ductus arteriosus in the present experiments, ranging from 39 to 53 per cent, agree with these figures.

The changes in the circulation immediately following occlusion of an experimental arterio-arterial fistula between the aorta and the pulmonary artery are of interest in connection with the surgical operation for ligation of patent ductus botalli in man. Dolley and Jones (1) emphasized the importance of first occluding the ductus arteriosus temporarily and watching for untoward effects on the cardiac action before proceeding with the ligation. Although the changes in the circulation which followed occlusion of an experimental ductus arteriosus did not result in the death of any of the experimental animals, the slowing of the pulse, increase in systemic blood pressure, decrease in cardiac output and decrease in oxygenation of mixed venous blood from the pulmonary artery are easily observed effects. The anesthesia employed should be one which insures an adequate intake of oxygen for if the subject were anoxemic or in an otherwise impaired condition, the effects of occlusion of the ductus might be harmful. In the light of these considerations, the technique advocated by Dolley and Jones is undoubtedly advisable.

SUMMARY

1. A condition similar to patent ductus botalli was produced in dogs by lateral anastomosis of the aorta to the left pulmonary artery and by end-to-side anastomosis of the left subclavian to the left pulmonary artery.

2. Studies were made of both the immediate and late effects of production of the experimental ductus arteriosus on the cardiac output, pulse and content of oxygen in the blood.

3. A study was also made of the immediate effects of occlusion of chronic experimental ductus arteriosus on the pulse, systemic blood pressure and that of the pulmonary artery and the cardiac output.

4. In the experiments of long standing the flow of blood through the experimental ductus arteriosus was calculated.

5. The significance of the findings as related to surgical ligation of patent ductus botalli in human patients is suggested.

REFERENCES

(1) DOLLEY, F. S. AND J. C. JONES. In Medico-surgical tributes to Harold Brunn. Univ. of California Press, Berkeley, 1942.

(2) EPPINGER, E. C., C. S. BURWELL AND R. E. GROSS. J. Clin. Investigation 20: 127, 1941.

(3) FICK, A. Sitzungsb. der phys.-med. Gesellach. zu Würzburg, p. 16, 1870.

(4) GROLLMAN, A. The cardiac output in health and disease. Charles C. Thomas, Baltimore, 1932.

(5) GROSS, R. E. AND J. P. HUBBARD. J. A. M. A. 112: 729, 1939.

(6) HARRISON, T. R., W. DOCK AND E. HOLMAN. Heart 11: 337, 1924.

(7) KEYS, A. This Journal 134: 268, 1941.

(8) LEVY, S. E. AND A. BLALOCK. J. Thoracic Surgery 8: 525, 1939.

(9) LEWIS, T. AND A. N. DRURY. Heart 10: 301, 1923.

(10) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative Clinical Chemistry. Vol. II (Methods), Williams & Wilkins Co., Baltimore, 1932.

THE SURVIVAL OF NON-ADRENALECTOMIZED RATS IN SHOCK WITH AND WITHOUT ADRENAL CORTICAL HORMONE TREATMENT

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In this study shock was induced in non-adrenalectomized rats by ligation of the two hind limbs. A comparison was made of the incidence of survival among rats which were treated with adrenal cortical extracts and steroids, and those which were untreated. The results of the study were negative.

METHODS. Normal male rats of the Sprague-Dawley strain, weighing 190 to 200 grams, were used. A short period of anesthesia was induced by the intraperitoneal injection of a solution of 18 mgm. of cyclopentenyl-allyl-barbituric acid sodium. The hair was clipped from the posterior half of the body. A ligature of strong linen thread was placed about each hind leg and held in place by four short stitches through the skin at the following points: 1, in front of the penis; 2, through the umbilical scar; 3, dorsal side, over the vertebral notch formed by the junction of the lumbar and thoracic vertebrae, and 4, just over the first caudal vertebrae. The ligatures were tightened alternately until they were as tight as possible. A second set of ligatures were then placed in the same positions but were not stitched through the skin. The ligatures were removed after two hours and thirty minutes. By this time the animals had begun to recover from the anesthetic. Food and water were withheld. At the time the ligatures were removed, the limbs were greatly swollen and congested with blood. Although the swelling and congestion were relieved to some extent following removal of the ligatures, the animals gradually lost the appearance of vigor and became prostrated. The incidence of survival for 24 hours was the criterion used to judge the therapy.

The adrenal cortical extract was prepared from beef adrenal glands. In experiment 1, each dose of extract was administered in 1.0 cc. of physiologic saline and each dose of the crystalline compounds was administered in 0.5 cc. of sesame oil. In experiment 2, an adrenal cortical extract, from which all traces of epinephrine had been removed, was administered in 0.5 cc. of sesame oil per dose. A control solution was prepared by dissolving physiologically inert material from adrenal extracts in sesame oil so that its appearance matched that of the active extract. The control animals received 0.5 cc. of this solution per dose. All test substances were given by subcutaneous injection at the time the legs were ligated and again six hours later.

The selection and matching of the rats with the test substances was by the following procedure: For experiment 1, a pack of cards, containing all the possible matchings of test substances with numbers from 1 to 5, was thoroughly shuffled. On each day a group of 5 rats were subjected to ligation of the limbs

and were then numbered from 1 to 5.   A card was drawn from the pack and the test substances were administered according to the matchings on the card.   A similar procedure was followed in experiment 2, with the added precaution that the experimental and control solutions were tested as "unknowns."

EXPERIMENTS AND RESULTS.   In experiment 1 the following substances were tested: adrenal cortical extract, corticosterone, 17-hydroxy-11-dehydrocorticosterone, 11-desoxycorticosterone acetate and a control solution of sesame oil. In table 1 the number of rats surviving out of each group of 20 is shown in relationship to the test substance, the size and number of doses.   The difference in the incidence of survival of the experimental and control groups was small and, in the case of the crystalline compounds, it is concluded that no beneficial effect was demonstrated.

In the case of adrenal cortical extract the results were suggestive of an increased resistance to shock.   However, since this extract contained traces of

TABLE 1

Relationship of treatment to the numbers of rats surviving out of each group of 20 animals

DOSE			TREATMENT				
Extract (gland equivalent)	Crystals	Number doses	Adrenal extract	E	Cortico	Doc-Ac	Sesame oil control
grams	*mgm.*						
20	0.25	1	7	4	5	7	5
40	0.50	1	9	8	10	10	7
40	0.50	2	12	9	8	9	6
80	1.00	2	14	11	10	9	10
160	2.00	2	11	7	11	10	9

E, 17-hydroxy-11-dehydrocorticosterone; cortico, corticosterone; Doc-Ac, 11-desoxycorticosterone acetate.   The term gland equivalent refers to the weight of the whole beef adrenal glands from which that dose of extract was derived.

epinephrine and was made up in physiologic saline, the favorable results may have been an artifact.

Experiment 2 was a study of an adrenal cortical extract from which all traces of epinephrine had been removed and which was given as a sesame oil solution. In table 2 the number of rats surviving in each group is shown in relationship to treatment.   The results of this experiment were negative.

DISCUSSION.   When the results of experiments 1 and 2 are considered together, it must be concluded that under the conditions of these experiments, the administration of extracts and pure steroids of the adrenal cortex failed to increase significantly the incidence of survival of rats in shock.   It should be emphasized that there are sufficient data from studies in this laboratory to show that these extracts and steroids rapidly exert their biologic effects when administered in sesame oil and that these biologic effects last for longer periods of time than were studied here.   Although in these experiments the test animals represented a homogeneous group, the experimental conditions were carefully standardized

and the comparison of matched treated and control animals was made simultaneously, the variability in incidence of survival among different groups was large. It is apparent that studies limited to the comparison of 15 to 20 pairs of test animals may give misleading results.

It has been reported by Selye et al. (1) and by Noble and Collip (2) that the resistance of non-adrenalectomized rats to shock caused by trauma was raised above normal by the administration of adrenal cortical hormones. There are a number of other reports on clinical and experimental observations in the literature which are relative to this problem but they will not be reviewed here. I am not aware of any data which prove conclusively that the resistance of the

TABLE 2

The relationship of the amount of adrenal cortex extract per dose to the number of rats surviving out of each group

SOLUTION	GLAND EQUIVALENT PER TWO DOSES	NUMBER TESTED	NUMBER SURVIVED
297	Control	20	8
298	80 gms.	20	6
297	Control	20	10
298	80 gms.	20	6
297	Control	15	0
298	80 gms.	15	9
288	80 gms.	20	7
289	Control	20	9
290	160 gms.	20	8
290A	Control	20	13
292	400 gms.	20	7
293	Control	20	7

The term gland equivalent refers to the weight of whole beef adrenal glands from which the combined (two doses) dosage per rat was derived.

non-adrenalectomized animal or of the patient lacking damage to the adrenal glands can be increased by the administration of adrenal cortical hormones. A consideration of the favorable reports of non-crucial experiments is of little value since there have been a number of experiments showing negative results which have not been published. At the present time the question must be left open as to whether the adrenal cortical hormones are capable of raising the resistance to stress of animals and patients having undamaged adrenal glands.

SUMMARY

Normal male rats of 190 to 200 grams weight were caused to develop shock by the ligation of the two hind limbs for a period of two hours and thirty minutes.

A comparison was made of the incidence of survival for 24 hours of rats which were treated with adrenal cortical extracts and with certain steroids and rats which were given control solutions. The results were negative.

REFERENCES

(1) SELYE, H., C. DOSNE, L. BASSETT AND J. WHITTAKER. Canad. M. A. J. **43:** 1, 1940.
(2) NOBLE, R. L. AND J. B. COLLIP. Quart. J. Exper. Physiol. **31:** 201, 1942.

THE ELECTROGRAM OF THE VENTRICLE OF THE TURTLE'S HEART

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Although the literature on the electric responses of ventricular muscle is extensive (see for references Katz, 1928, and Schütz, 1936) the pattern of the so-called "monophasic" records is still open to question. Indeed, in recent years the source of those records has been attributed to the damaged, rather than to the normal tissue (Wilson, Macleod, Johnston and Hill, 1933; Eyster, Meek, Goldberg and Gilson, 1938). The present study attempts an analysis of the pattern and site of origin of the ventricular electrogram of the turtle.

METHOD. After pithing, the heart of the turtle was excised. A quiescent ventricle was obtained by removal of the atrium. Three different ventricular preparations were employed. Two cuts were sometimes made along the two lateral ventricular edges, from the base to about 0.5 cm. from the apex. The muscle was then unfolded to yield a flat mass with a ridge in the center, corresponding to the apical region. It was laid with the inside surface downwards on filter paper moist with Ringer solution and attached to a board by pins. One corner was left free from fixation and was connected to a torsion-spring myograph. This preparation will be referred to as the "unfolded ventricle."

In other cases ventricular strips, about 5 mm. wide, were cut perpendicularly to the base. One end was fixed inside a moist chamber and the other was attached to the myograph. This preparation will be designated "ventricular strip."

In the majority of the experiments only electrical recording was desired. The intact ventricle was then placed on moist filter paper and fixed to a board by pins, as in the first preparation. After placement of stimulating and recording electrodes, the muscle was covered by a large glass funnel, which ensured a relatively closed moist chamber.

The electrodes were either chlorided silver needles, which rested lightly on the external surface of the ventricles, or else large chlorided silver plates connected to the tissue by an Agar-Ringer bridge followed by a moist wick.

Single or multiple electrograms were recorded by cathode-ray oscillographs. Direct-coupled amplifiers were used, so that the responses were recorded without distortion. The input impedance of the amplifiers was high (1 or 2 megohms). The records correspond therefore mainly to differences of potential. The procedure followed when recording with one lead corresponding to the stimulating cathode has been described elsewhere (Rosenblueth, Daughaday and Bond, 1942).

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When both mechanical and electrical tracings were desired, the beam of light from the myograph was sent to the back of the film which photographed the single oscillograph used in those observations.

The stimuli were condenser discharges with variable voltage and with a brief time constant (about 0.8 msec.). The stimulating cathode was always on intact tissue.

RESULTS. A. *Electrical responses recorded from intact to injured ventricle.* A characteristic electrogram recorded from an intact to a crushed or burned region is illustrated in figure 1A. The pattern is complex, that is, it exhibits several crests and troughs. As indicated by the numbers which label the tracing, four excursions in which the intact region becomes increasingly electronegative (*1, 3, 5,* and *7*) are separated by three positive dips, two (*2* and *4*) relatively,
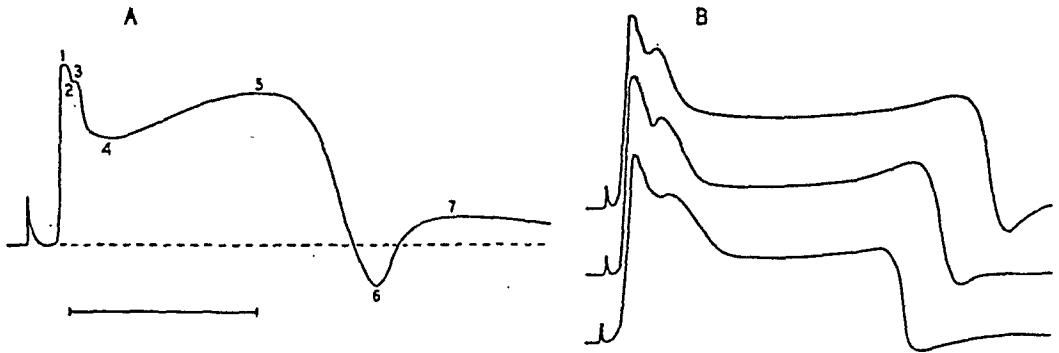
Fig. 1. Tracings of responses from leads on crushed and intact regions of a ventricle. In this and other figures with leads from live and injured tissue, respectively, upward excursions denote negativity of the lead on live tissue. The horizontal line corresponds to a time interval of 1 sec.

A. Characteristic electrogram. The numbers label the successive changes of potential differences. The record shows atypically prominent excursions 4 and 7.

B. From above downwards, the 1st, 4th and 7th responses to a series of stimuli at the rate of 1 per 3 sec.

and the other (*6*) absolutely positive with respect to the preexisting demarcation potential.

All the waves which appear in figure 1 were not invariably present in the records. Excursion *7* was often unidentifiable. Less frequently the troughs *2* and *4* were absent. Even more rarely the positive dip *6* was absent. The records then approached the theoretical monophasic single negative wave which has often been assumed to represent the pure ventricular electrogram. Since all the excursions in figure 1 were usually recognizable, however, even when the records were taken immediately after crushing or burning the tissue, they should be included in the detailed description of a typical electrogram.

A complete electrogram, similar to that illustrated in figure 1, was found in the cat's ventricle, as follows. In three cats under dial anesthesia artificial respiration was administered. The thorax was opened by resection of the sternum. The apex of the heart was tied tightly and the ligature was fixed to a clamp, to reduce movement. The records from a lead on the tied (injured)

region to one at the base of either ventricle showed all the excursions in figure 1 as soon as tested—i.e., within 2 minutes after the injury. An additional brief diphasic excursion preceded the component *1*. This excursion is attributed to the propagation of the impulse in the conducting tissue, for it varied when ectopic beats were obtained during vagal stimulation, while the rest of the electrogram was not significantly altered.

Additional excursions were sometimes seen in the turtles. The most common was a slight initial positivity or negativity, which preceded the negative swing *1* in records taken after conduction—i.e., at some distance from the stimulating cathode. Since this initial deflection was never seen in records from the cathode, and since it was not always present in records after conduction, it is inferred that it is not an essential constituent of the electrogram.

Repetitive stimulation at a rate of 1 per 2 or per 3 secs. led to striking changes of the electric responses. As illustrated in figure 1B the responses became briefer
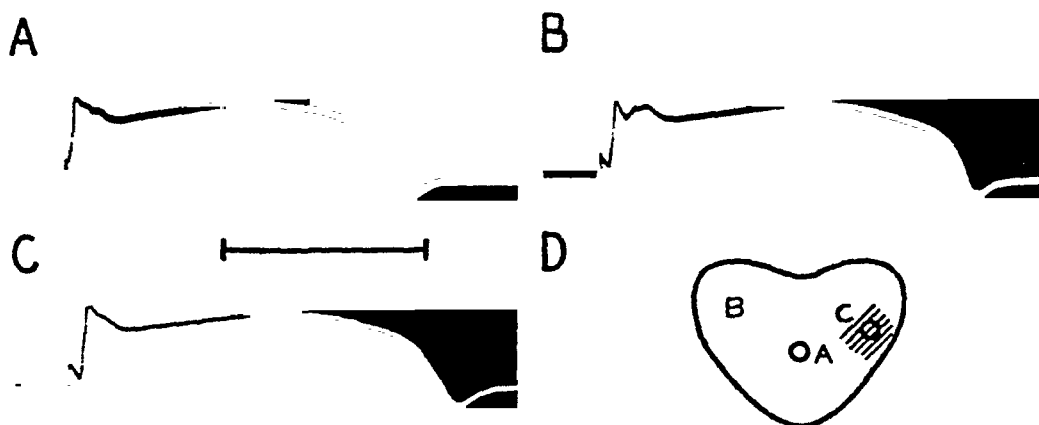
Fig. 2. Invariance of the responses obtained from an intact to a crushed ventricular region.

A, B and C, responses to stimuli with the cathode placed at the positions indicated by the corresponding letters in the diagram D. The hatched part of the diagram indicates the crushed region and the circles the position of the leads.

with successive shocks. This shortening was mainly at the expense of the late components of the electrogram, especially excursion *5*. Indeed, the early components, particularly *2* and *3*, occurred later than in the first response, and were prolonged. An early appearance of the positive wave *6* is the most striking feature of the brief responses.

The pattern of the electrograms was not significantly affected by the path of conduction followed by the propagated response in its travel towards the recording electrodes. Thus, the records in figure 2 were obtained from leads in a fixed position, but with the stimulating cathode at the points illustrated in the diagram. The responses are quite similar regardless of the region stimulated.

The latency of the responses was found to increase with the distance between the stimulating cathode and the lead on intact tissue. Since the stimulus-response delay is variable and may be quite long in the turtle's ventricle (Rosenblueth, Daughaday and Bond, 1942), the following procedure was adopted for

these observations. Simultaneous and independent records were taken from a common lead on injured tissue to each of three other leads on intact ventricle. For any given response the latency was shorter in the record from the lead nearer the stimulus. Figure 3 illustrates a typical experiment.

The latency of the responses was not influenced by the distance between the stimulated and the injured regions. Thus, in figure 4 the latency of the responses recorded from three crushed regions, respectively, to a common lead on intact tissue is the same for the three records, although the stimulus was applied close to one of the crushes and relatively far from the others.

The duration and pattern of a given response varied for several positions of the leads on live tissue (figs. 3, 7 and 10). Small, brief and atypical responses were usually recorded from leads placed at a short distance (1 to 4 mm.) from the injured region (see figs. 4B, 7B, and 10). On the other hand the amplitude

Fig. 3   Fig. 4
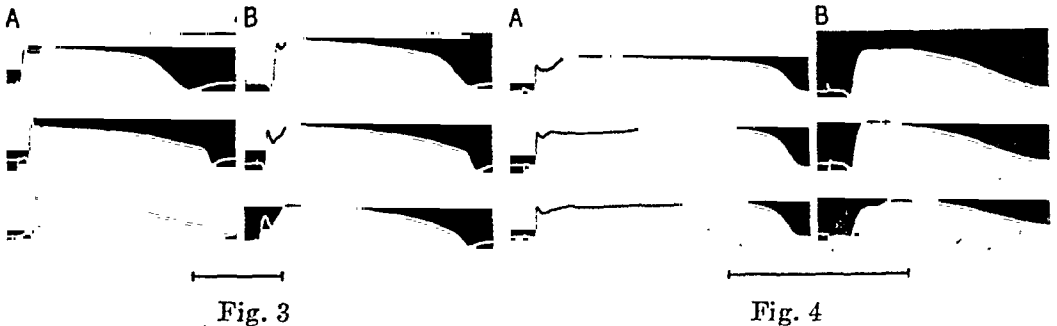
Fig. 3. Independence of the records taken from a common lead on burned tissue to three different leads on intact ventricle. The live leads corresponding to the records from above downwards were 3, 6 and 9 mm. away from the burn, respectively.

In A the stimulating cathode was 1 mm. away from the lead for the top record, in B it was near the lead of the lowest record.

Fig. 4. Similarity of responses recorded from one common lead on live tissue to different leads on three separate burned regions. The stimulating cathode was near one of the burns. In A the live lead was relatively far from all the injured points, in B it was 2 mm. away from one of the burns.

duration and pattern of a given response were practically constant when a common lead on intact ventricle was tested against several crushed regions (fig. 4).

B. *Electrical responses recorded from two intact regions of the ventricle.* As opposed to the records from intact to crushed tissue, which had a similar pattern both for a given ventricle and also from one ventricle to another, the records from intact to intact tissue were quite variable. Several factors could influence their pattern. The typical record, with the two leads about 1 cm. apart and aligned with respect to the stimulating cathode, consisted mainly of two waves, one initial, the other terminal (see fig. 5). In rare simple cases each of these waves was monophasic, the first showing electronegativity and the second electropositivity of the lead proximal to the stimulus with respect to the distal lead. More commonly, however, both waves were complex, di- or even triphasic, and their polarity, particularly that of the terminal one, was variable.

The two main factors which modified the pattern of the records were the orien-

tation of the leads with respect to the wave-front of the propagated impulse and the recording interelectrodal distance. The more equidistant the two leads from the stimulating cathode, and the shorter the distance between them, the briefer the first wave of the record.

The second wave was often modified in the course of a series of responses elicited with relatively fast rate of stimulation and recorded with fixed leads. This wave then usually became briefer and its polarity could sometimes be reversed during the series. The phenomenon is illustrated in figure 6.

The polarity of the second wave was usually unchanged when stimuli were delivered first near one then near the other recording lead. In other words with fixed leads a and b, a could be negative with respect to b during the second wave whether the stimulating cathode was proximal to a 'or to b (fig. 5). The first wave, on the contrary, always reversed in these circumstances—i.e., the lead proximal to the stimulus was mainly negative with respect to the distal (fig. 5).
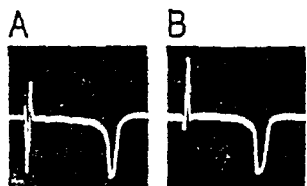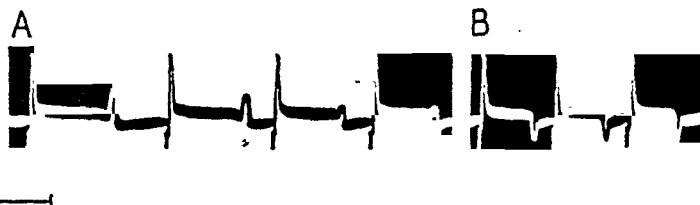
Fig. 5   Fig. 6

Fig. 5. Changes of the records from two leads on intact tissue produced by stimulating different regions of the ventricle. One lead near the base, the other near the apex. Upward excursions denote negativity of the apical, with respect to the basal lead. At A the stimulating cathode was near the base, and at B near the apex. The stimulus artifact was too small and does not appear in the records.

Fig. 6. Changes of the records from two leads on intact tissue in the course of a series of responses. Both stimulating and recording electrodes were in fixed positions. A, beginning, and B, 8 sec. later, end of series.

Records from intact to intact tissue have commonly been attributed to the algebraic summation of the electrical events taking place under each of the leads. Figure 7 shows responses recorded between two leads on intact tissue and from these to another at a crushed region. It is quite clear that the record from live to live tissue is the algebraic sum of the other two. Indeed, when the records were projected through a photographic enlarger and corrected for the slight differences in the gain of the respective amplifiers an accurate summation was obtained.

This method afforded a means to test how strictly similar were the responses from a lead on live tissue to two different crushed regions. If the two records were quite similar no deflections should be recorded between the two leads on crushed tissue. Although as a rule there were slight differences, occasionally, as shown in figure 8, the two electrodes on dead tissue remained practically isopotential throughout a response, even when the stimulus was much closer to one of the crushed regions than to the other.

C. *So-called unipolar leads.* The method of recording from one localized lead on the surface of the ventricle to another electrode in the body of the animal or in the Ringer solution partially bathing an excised heart was used by Wilson *et al.* (1933) and by Eyster *et al.* (1938). It was considered interesting, therefore, to compare the results from this mode of recording with those reported above.

All our observations agree with the inference (see discussion below) that the external electrode is equivalent to a diffuse lead applied to a large fraction of the ventricular surface. The influence of the position of the leads on live tissue with respect to the stimulated region was less striking with the external leads (figs. 9 and 10) than with those applied to a localized region (fig. 3), as would be expected from a diffuse lead, but it was clearly recognizable.

Fig. 7   Fig. 8   Fig. 9
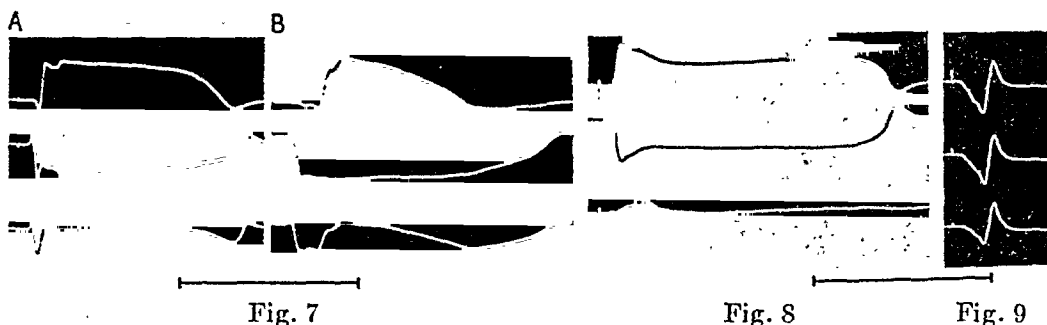
Fig. 7. Algebraic summation of two records from one injured to two intact regions yields the record from intact to intact regions. The two upper records correspond to a lead at a burned region and each of two separate leads on live tissue. The lower record is from the two leads on intact tissue. In A both live leads were well away from the burn. In B the lead corresponding to the upper record was near the burn.

Fig. 8. Negligible differences of potential recorded from two leads on burned ventricle although the stimulus was applied close to one and far from the other burn. The two upper records are from each of the burns to a common lead on live tissue. The lower record is from the two burns.

Fig. 9. Differences between records obtained from a common localized lead on live ventricle to 3 different electrodes placed in contact with Ringer solution bathing the heart partially. For explanation see text.

In figure 9, three external leads are tested against a single localized lead on live tissue. Only the beginning of the records is shown. The upward excursion, simultaneous in the three records, signals the arrival of the impulse at the localized lead. The external lead corresponding to the top record was on the side of the heart proximal to the stimulated region. The initial downward excursion is more prominent in this record. The lowest record is from the external lead on the opposite side of the ventricle. The initial downward excursion is slow and small. The middle record, from an external lead in an intermediate position, shows likewise intermediate features of the first excursion.

In figure 10 a lead on burned tissue is referred to an external lead (top record) and to two localized leads on intact tissue, one (middle record) 3 mm. distant from the burn, and the other (lowest record) 6 mm. away. The stimulus was applied 15 mm. away from the burn. The three records differ in latency, in pattern and in duration.

D. *The influence of veratrine on the electrogram.* The negative after-potentials of nerve (Graham, 1930), of the superior cervical ganglion (Rosenblueth and del Pozo, 1942a) and of striated muscle (Rosenblueth, Wills and Hoagland, 1941), are all markedly increased by veratrine. Observations were made, therefore, to see whether any of the components of the ventricular electrogram would be similarly increased by this drug, thus indicating an analogy with the negative afterpotential of the structures mentioned.

Veratrine (0.5 to 1 mgm.) was injected into the atrium in some animals, before excision of the heart. Although the doses of the drug used were sufficient to cause the appearance of rhythmic activity in some of the ventricles tested, in no case was there a significant increase of any of the components of the electrogram. The records from the veratrinized ventricles were quite similar to those obtained from the normal muscles.
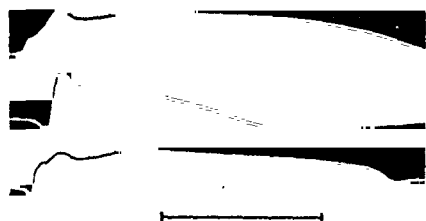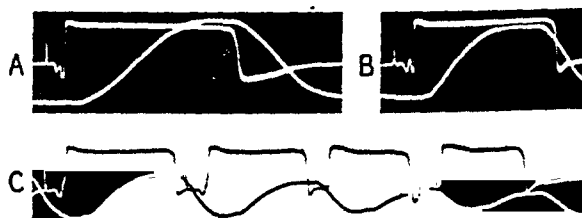
Fig. 10.   Fig. 11.

Fig. 10. Differences in the records from a lead on burned tissue referred to an external lead on the Ringer solution partially bathing the preparation (top record) and to two localized leads on intact tissue, respectively. One of the latter electrodes (middle record) was 3 mm. away from the burn, the other (lowest record) 6 mm. away.

Fig. 11. Changes in the electrical and mechanical responses in the course of a series of stimuli. Unfolded ventricle (see Method). Upper record: electrogram; lower record: isometric mechanogram. One of the recording leads was on the part of the ventricle which registered mechanically, the other lead was on a crushed part, 5 mm. away. A, 1st response; b, 4th response; and C, the last 4 responses in a series of 10.

Similarly negative results were found in the three cats studied (p. 465). Injections of veratrine (1 to 2 mgm. per kgm.) did not modify the electrograms significantly.

E. *The relations between the electrical and mechanical responses.* Since this is a point which has been abundantly studied (see Schütz, 1936), only incidental observations were made, with the limited purpose of seeing whether any of the components of the electrogram would show variations parallel with those in the mechanical events.

Efforts were made to obtain the electrical records from the region of the ventricle which was registering mechanically. This was not feasible in the strips of muscle, since the electrical record was relatively localized, whereas the whole strip contributed mechanically. In the unfolded ventricles (see Method), on the other hand, the contractions recorded were mainly those from a small part of the ventricle and the electrical responses were led from one electrode on that part and the other about 0.5 cm. away on a crushed region.

The beginning of contraction coincided usually with the excursion *3* (fig. 1) of the electrogram. There was no correlation, however, between the beginning of relaxation and any of the electrical events. Indeed, when a series of beats was elicited at rates of 1 per 2 to 1 per 5 secs. the electrical and mechanical responses varied independently. As shown in figure 11, both electrical and mechanical events became briefer, but the contractions shortened more at first whereas the electrograms shortened more than the mechanograms later in the series.

The only electrical phenomenon that showed any correlation with the amplitude of contraction was the positive swing *6* (fig. 1). This correlation was very striking in the only two ventricles studied which exhibited alternation of contractions when stimulated at a rate of about 0.7 per sec. The large mechanical responses coincided with a large *6* in the electrogram, whereas the small contractions were attended by only a small *6* (fig. 12).

Fig. 12. Alternation of the mechanical responses after veratrine (0.5 mgm. into the auricle, before removal of the heart). Lower tracing: mechanogram. Upper tracing: monotopic electrogram. Note the alternation of component *6* (see fig. 1). The record begins with the second stimulus in a series.

DISCUSSION. I. *Some problems of electrical recording.* The terms monophasic and diphasic are often employed to distinguish records obtained from intact to damaged tissue and those led from two intact regions. As has often been pointed out, these terms are ambiguous. Strictly speaking a monophasic record is one exhibiting a single unidirectional wave. No tissue has so far yielded such a record. The so-called monophasic record of nerve exhibits several components, some negative, some positive (see Gasser, 1937); it is therefore not a monophasic, but a polyphasic record.

A fundamental question in the study of the electrical behavior of any cell or tissue is the determination of the potential changes during activity at an ideally isolated small region. These changes can be referred to a local system of coordinates, e.g., from inside to outside the cell, and also to an ideal indifferent lead on inactive tissue. The term monopolar, often used to characterize a mode of stimulation, might be used to describe the records which would answer that question. But the term monopolar is a misnomer, for records, like stimuli, require two poles—two electrodes. We propose, therefore, to use the term "monotopic" to designate a record which is representative of electrical events at a small region of a tissue.

II. *The site of origin of the electrical changes recorded from an intact to an injured region.* The classical view is that those records are due to changes in the intact region of the ventricle to which the lead is applied. As already mentioned,

however, Wilson *et al.* (1933) and Eyster *et al.* (1938) have suggested that the source of the potential changes is the damaged region.

This suggestion is based mainly on observations made with so-called unipolar records. As pointed out (see figs. 9 and 10), however, this procedure is equivalent to leading directly from one localized to another diffuse lead on the ventricle (p. 469). The external lead is not an indifferent lead. At best it indicates the average potential, but this average can fluctuate widely depending on the electrical events in the tissue.

The following arguments are against the view that the injured tissue, or the immediately neighboring regions, are the source of the responses recorded. The same arguments support the opposite view that the source is the intact muscle. It is highly improbable that dead tissue should respond, even when attached to live tissue. In analogy with what occurs in nerve, one would expect not only that the dead tissue should be inactive, but also that the muscle in the neighborhood of the injured region should be depressed. In accord with these expectations, when records are taken with two localized leads, the closer the electrode on live tissue is to the injury, the smaller and briefer the response (see figs. 4B, 7B and 10).

If the responses with localized leads from injured to intact regions were mainly due to events at or near the damaged area (Eyster *et al.*, 1938), then the records from one injury to different intact points should have a constant latency and duration, regardless of the point stimulated, but this is not the case (figs. 3, 7 and 10). On the other hand, significant differences of latency and duration would be expected when leading from several injured regions to one intact portion, but this is also not a fact (figs. 4 and 8). When the stimulus is applied near one of several injured portions and leads are taken from two of them, asynchronism would cause marked deflections in the record if the source of the responses were at the injury, but the records exhibit only minor differences of potential (fig. 8). Finally may be mentioned the report of Schütz (1936, p. 515) that local warming of the injured region has no influence on the recorded responses, while local warming of the intact region to which a lead is applied causes the responses to become markedly briefer.

It is inferred, therefore, that in the heart, as in nerve and skeletal muscle, the electric responses originate from intact, not from damaged parts.

III. *The monotopic character of the records from intact to dead tissue.* Although, as shown above, the electric responses originate from intact tissue, the previous argument does not answer the question of what parts of the tissue contribute to the records—i.e., whether it is only the intact region in contact with the lead that records, or whether other uninjured portions of the ventricle are also contributory.

That the records are mainly monotopic, from the intact portion tapped, is shown by the following data. The independence of latency, amplitude and duration of records from different live spots (fig. 3) is in favor of that view. The invariance, except for latency, of responses recorded from fixed leads and variable stimulated regions supports also the view (fig. 2). Finally, Schütz's (1936)

observations on the action of local heating, cited above, are favorable to the inference.

IV. *The components of the monotopic response.* It has often been assumed that this response should be strictly monophasic—i.e., should consist of a single, simple, unidirectional excursion—and that any deviations from that simple tracing are due to artifacts. The actual records seldom, if ever, conform with this ideal pattern. Their complexity has been recognized by the distinction of two components (see for references Katz, 1928, and Schütz, 1936). To account for the 7 excursions illustrated in figure 1, at least 4 components must be assumed, if those excursions are not artifacts.

Spurious waves could be due to two factors: movement artifacts and an asynchronous contribution of similar, relatively simple events at different regions of the muscle, specifically the regions in the neighborhood of the lead on crushed tissue (so-called diphasic artifacts).

The early part of the electrogram (excursions *1* and *2*) preceded the mechanical response if the lead on intact tissue was sufficiently close to the stimulating cathode. This part of the electrogram took place before the propagated impulse reached the neighborhood of the lead on crushed tissue if the recording interelectrodal distance was large and the stimulus was applied far from the crush. Since records similar to that in figure 1 were obtained with relatively large interelectrodal distances (about 2 cm.) and with stimulation at or near the lead on intact tissue it may be inferred that the early part of the electrogram is free of artifacts from the two sources mentioned.

Both movement and asynchronism should be modified if, with a fixed position of the recording leads, the cardiac responses were started at different points by moving the stimulating electrodes. Hence, if the two sources of artifact mentioned were significant, shifts of the stimulating electrodes should significantly modify the recorded electrogram. It was found, however, that the electrical responses did not as a rule vary when the stimulated region was varied (fig. 2).

Movement artifacts may occur. Thus, it is likely that the initial deflection seen sometimes in responses recorded after conduction (p. 466) was due to movement. The responses from a given pair of leads usually changed slowly in the course of time, the main feature being the accentuation of the positive component *4*. Further crushing or burning in the injured region then restored the original pattern. This change with time is therefore probably due to progressive healing of the injury—i.e., it constitutes a "diphasic" artifact.

Since all the components in figure 1 were usually present within 1 minute after injury and in approximately isometric conditions, it is concluded that these components are genuine constituents of the monotopic electrogram. It would be surprising if the ventricular response were simple, for in all the tissues studied so far—nerve (see Gasser, 1937), striated muscle (Rosenblueth, Wills and Hoagland, 1941) and smooth muscle (see Rosenblueth and del Pozo, 1942b)—the electrogram is complex.

The conducting mechanism in the turtle's ventricle is similar in its properties to that in nerve and in striated muscle. The first component (*1*) in the ventric-

ular electrogram may therefore be analogized with the spike potential of the other tissues. Since the positive component *6* of the ventricle was found to vary with the degree of contraction (fig. 12), this component may be analogous to the positive component which varies in striated muscle with contraction (Rosenblueth, Wills and Hoagland, 1941). No further analogies may be drawn, however, with the data available. A puzzling item of negative information is the relative indifference of the ventricular electrogram to veratrine. Since in other tissues (see p. 470), a spike potential is followed by a negative afterpotential which increases dramatically with veratrine, the data on the heart may imply that the ventricle lacks this type of negative afterpotential. It is possible, however, that this potential may exist, but that even when increased by veratrine it is masked by other, more prominent, negative components (*3* and *5*).

V. *The records from intact to intact regions.* Since the records from live to injured regions are monotopic and correspond to the live point, it follows that the records from live to live leads are ditopic and correspond to the algebraic summation of the events at each of the two leads. This corollary is illustrated in figure 7. It should be pointed out, however, that an accurate algebraic summation of the potential changes at several electrodes forming a closed loop (fig. 7; see Eyster *et al.*, 1938) does not prove anything with regard to the source of the potentials recorded. What it proves is merely that Kirchhoff's e.m.f. law applies to the cardiac circuit, much as it applies to any other circuit which has been tested so far. In other words, the potential changes between any two electrodes on the heart will always be the algebraic sum of the changes between these two leads and any number of other electrodes, so arranged that they form a closed loop. The applicability of Kirchhoff's law does not give any information about the location of sources of differences of e.m.f.

The analysis of the factors which determine the terminal wave of the ditopic elelectrogram is of significance for the interpretation of the wave T of the electrocardiogram. The data indicate that the most important factor is the asynchronous termination of the electric responses at the two recording regions. This asynchronism might be due to the discrepancy of the beginning of the response at the two points, because of the time lost in the conduction of an impulse from one to the other. The results suggest, however, that conduction time is a relatively unimportant factor for the determination of the pattern and polarity of the terminal wave (figs. 5 and 6). The most important condition, apparently, is the difference in the duration of the action potentials at different regions of the ventricle. Not only is this duration variable for different regions, but it may vary independently at each of them in a series of responses (fig. 6).

The complexity and variability of pattern of the terminal wave of ditopic records is readily understood since the terminal part of the monotopic records is itself complex (components *5, 6* and *7*). The algebraic summation of two asynchronous simple waves would lead to a relatively simple pattern, but the summation of complex events can lead to an intricate sequence of potential changes.

VI. *The relations between the electrical and mechanical events.* Although the

observations on the mechanical responses were only incidental, some comments are justified by the data obtained.

The correlation between the time course of mechanical and electrical events in figure 11 can only be approximate, because the electrical record is monotopic whereas the mechanical record corresponds to a relatively large portion of muscle whose elements respond asynchronously. Notwithstanding this lack of accuracy, the changes during a series of responses support the conclusion that the electrical and the mechanical phenomena are largely unrelated. Many other lines of evidence lead to the same conclusion (see Schütz, 1936).

Blair, Wedd and Young (1941) have proposed the hypothesis that the contractile process is released by the depolarization wave consequent to excitation and proceeds as long as the membrane remains depolarized. The contractile process would be retarded or arrested when the membrane began to repolarize. The arrest would be complete by the time repolarization is complete. Thus polarization would govern contraction, not contraction polarization. The present observations do not support this hypothesis. If polarization (i.e., negativity of the active region) controlled contraction, then there should be a strict temporal correlation between the end of that negativity (component *6*) and the beginning of relaxation. But figures 9 and 10 show that this correlation does not exist.

It is concluded that the potential changes and contraction are largely independent. The spike potential (component *1*) may not only be the agent for conduction but may also trip the contractile process. The time course of contraction is probably governed by other factors than the potential changes. It is likely that one (component *6*) or more of the excursions in the electrogram (fig. 1) are the electric manifestation of the physicochemical events associated with contraction; in that sense contraction may control polarization. It is also likely, however, that several of the electrical changes denote processes irrelevant to contraction—i.e., they may be associated with conduction and its recovery cycle.

SUMMARY

Evidence is presented (figs. 2, 3, 4, 8, 9 and 10) which indicates that the electric phenomena recorded from leads on an intact and an injured region of the turtle's ventricle are due to changes which occur in the intact, not the injured part of the muscle (p. 472). This record may be called monotopic, to avoid the use of other ambiguous terms (p. 471). This electrogram exhibits several components (fig. 1A), which may vary independently (figs. 1B and 12). The significance of some of these components is discussed (p. 473).

The records with leads from intact to intact tissue are influenced by the position of the leads with respect to the stimulated region (fig. 5). They may also be modified in the course of a series of responses (fig. 6). These records may be called ditopic, since they represent the algebraic summation of two monotopic records (fig. 7, p. 474).

The electric and the mechanical phenomena of the ventricle are largely independent (fig. 11, p. 475).

REFERENCES

BLAIR, H. A., A. M. WEDD AND A. C. YOUNG.   This Journal 132: 157, 1941.

EYSTER, J. A. E., W. J. MEEK, H. GOLDBERG AND W. E. GILSON.   Ibid. 124: 717, 1938.

GASSER, H. S.   In J. ERLANGER AND H. S. GASSER.   Electrical signs of nervous activity. Philadelphia, 1937.

GRAHAM, H. T.   J. Pharmacol. and Exper. Therap. 39: 268, 1930.

KATZ, L. N.   Physiol. Rev. 8: 447, 1928.

ROSENBLUETH, A., J. H. WILLS AND H. HOAGLAND.   This Journal 133: 724, 1941.

ROSENBLUETH, A., W. DAUGHADAY AND D. D. BOND.   Ibid. 138: 50, 1942.

ROSENBLUETH, A. AND E. C. DEL POZO.   Ibid. 136: 699, 1942a. Ibid. 137: 263, 1942b.

SCHÜTZ, E.   Ergebn. d. Physiol. 38: 493, 1936.

WILSON, F. N., A. G. MACLEOD, F. D. JOHNSTON AND I. G. W. HILL.   Proc. Soc. Exper. Biol. and Med. 30: 797, 1933.

TONUS CHANGES IN CARDIAC MUSCLE AND THEIR SIGNIFICANCE FOR THE INITIATION OF IMPULSES

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In a previous paper non-conducted potential changes were described which can initiate impulses and are responsible for the automaticity of cardiac muscle (2). Here it will be shown that these electric changes are accompanied by active changes in tension which, like the former, are non-conducted and graded. Because they are quite distinct from the all-or-none responses they will be called tonus changes, but they have nothing in common with the well-known tonus waves of the turtle's auricle.

METHODS. Most experiments were carried out with the ventricles of turtles. Responses were elicited by induction shocks. The contractions of muscle strips (3 to 4 mm. wide, 1 cm. long) were recorded by a very sensitive isometric lever on a smoked drum or photographically. The preparations were immersed in Ringer's solution except for the upper tip to which one of the stimulating electrodes was attached. The responses of the whole ventricle were recorded under isometric conditions by a membrane manometer on a smoked drum. The manometer was connected to the ventricle by a glass tube inserted through the auricle. The A-V valves were removed and the large arteries were tied off at their origin. The whole system was filled with fluid and a side tube was provided for regulating the initial pressure. The natural period of the whole system was 12 vibrations per second. The sensitivity of the manometer was high at low pressure and decreased at higher pressures which made the instrument particularly suitable for recording weak tonus changes.

For simultaneous recording of tension and potential the muscles were suspended in a shielded chamber above the muscle lever. The upper end of the muscle was inside a glass tube so that monophasic potentials could be recorded (1, 2).

RESULTS. The tonus changes following a conducted response were studied in two series of experiments. In the first series, the contractions of strips from the ventricle were recorded by an isometric lever. In the second series the contractions of the whole ventricle were recorded by a manometer. The results of both series were essentially the same, showing that mechanical injury like that involved in the preparation of the strips is not a necessary condition for the tonus changes. It was found that the tonus changes are very similar to the after-potentials previously described, a rise in negativity corresponding to a rise in tonus and vice versa.

In a fresh preparation the tension of the muscle drops below the previous resting level after each contraction (fig. 1A, 2A). This phenomenon, which corresponds to the positive after-potential present under the same conditions

(2), is significant because it shows that a weak tension is actively maintained even in resting muscle. The transitory drop in tonus was found in all experiments with muscle strips although its magnitude was very variable. It was absent in two of the twelve whole ventricles used and in two others it was found only when the initial pressure was higher than 6 mm. Hg. The changes in ten-

Fig. 1. Tonus changes following responses of a strip from the turtle ventricle recorded by an isometric lever. A. Muscle in Ringer's showing decrease of tonus below previous resting level and slow return after response. B. Same muscle in mixture of equal parts of 1 per cent $CaCl_2$ and Ringer's. Temp. 23°. Calibration 2 grams. Time seconds.
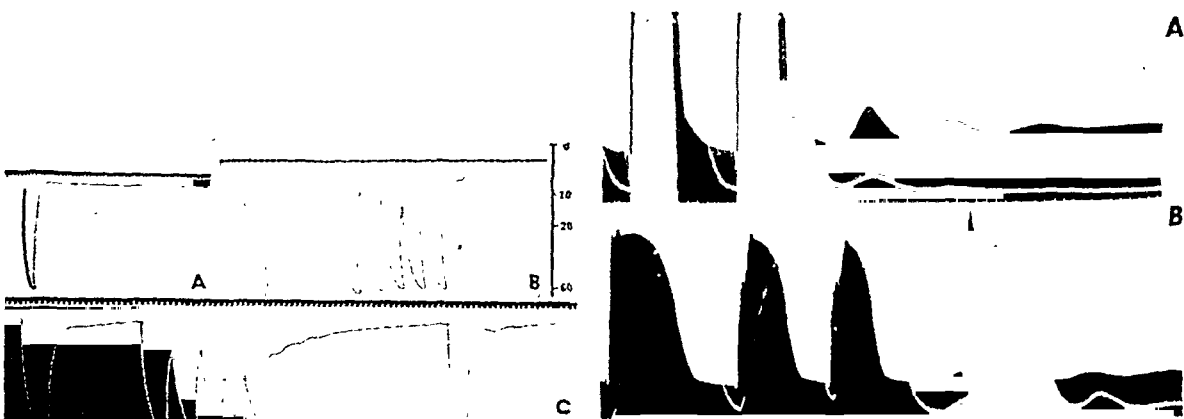
Fig. 2   Fig. 3

Fig. 2. Tonus changes of a whole ventricle recorded by a manometer. A. Ventricle filled with Ringer's, showing decrease of tonus after response. B. Ventricle filled with equal parts of 1 per cent $CaCl_2$ and Ringer's showing oscillatory after-effect. C. Same as B but with higher initial pressure giving a slower relaxation. Calibration: mm. Hg pressure. Note decrease in sensitivity of manometer at higher pressures. Time seconds.

Fig. 3. Oscillatory after-potentials and tonus changes recorded simultaneously from a strip of the turtle's ventricle. Upper curve oscillograph record, lower curve isometric lever. All-or-none responses were elicited by induction shocks except the last response in B, indicated by +, which was initiated by a local potential and was preceded by a tonus change. Time ½ second.

sion or pressure following the conducted responses were always very small, but no mechanical defect of the recording system was found which could account for the observed phenomena. Slipping or leakage would have produced a drop in tension or pressure, but could not explain the subsequent rise. The lever used had a small elastic after-effect which tended to diminish the effect recorded.

After treatment with a large excess of calcium ions the tonus becomes elevated

and relaxation is slower than normally, particularly at high initial tensions. In some cases, the tension drops markedly below the previous level after a response. The most striking effect of this treatment, however, are rhythmic variations of pressure or tension which decrease in a regular manner and disappear after 1 to 5 waves exactly like the oscillatory after-potentials described previously (fig. 1B; 2B, C; 3). The electric and mechanical changes run closely parallel but, as expected, the former always slightly precede the latter (fig. 3). Tonus oscillations have also been recorded from preparations of the sinus venosus. They are quite distinct from the tonus waves caused by smooth muscle.

Because of technical difficulties it has not been tested whether the spontaneous prepotentials such as they occur in the sinus venosus are accompanied by tonus changes. However, this is probably the case because there does not seem to be any essential difference between pre- and after-potentials and because in the ureter the tonus changes accompanying spontaneous prepotentials have been demonstrated.

DISCUSSION. It is not intended to give a full discussion of the question of tonus in cardiac muscle. The term is generally used to describe changes in "resting length" or, in other words, slow contractions which are sharply distinguished from all-or-none responses. Several investigators have claimed to have demonstrated such a phenomenon in cardiac muscle, but it appears that the underlying observations can be explained as the result of variations in the completeness of relaxation between beats caused by changes in heart rate or in the strength and duration of each beat (cf. 4). Such an interpretation cannot be applied to the tonus changes described above because single responses of quiescent muscles were studied.

It has been shown here that a small tension is actively produced and maintained even in the "resting" muscle. Obviously, this tension could not have been distinguished from passive elastic tension as long as it remained constant. The presence of a tonic contraction, therefore, cannot be detected by a study of the mechanical properties of the muscle alone and the question whether a similar condition exists also in other striated muscles can only be decided by further studies.

The tonus changes described may be considered as changes in the "resting" tension of the muscle. There is no reason, however, to assume a fundamental difference between this type of contraction and all-or-none responses. Both are accompanied by potential changes. The difference probably lies in the excitatory mechanism and chiefly in the presence or absence of conduction. That the tonus changes involve the production of mechanical energy is shown most strikingly in the tonus oscillations. It must be assumed, therefore, that tonus changes are caused by changes of metabolism. A simple explanation of the phenomenon would be the assumption that the resting metabolism involves the contractile mechanism and thereby causes the production of a small amount of mechanical energy. This assumption, according to which there is only a quantitative difference between activity and resting metabolism, is not in disagreement with our present knowledge of the chemistry of these processes.

The oscillations of tonus and electric potential undoubtedly express an important property of cardiac muscle, but their nature is obscure. Although they resemble oscillations of simple systems, a purely physical explanation based on mechanical and electric forces operating at the cell surface, such as the hypothesis considered by Cole (3) for potential oscillations in nerve fibers, appears untenable. The fact that in muscle mechanical changes are associated with the oscillations shows that they do not involve merely the cell surface. Their low frequency, several hundred times smaller than in nerve fibers, adds another difficulty for the application of Cole's hypothesis to muscle. The frequency in the various tissues studied so far appears to be correlated with the time relations of their responses. The phenomenon is perhaps comparable to chemical oscillations.

SUMMARY

The after-potentials of cardiac muscle previously described are accompanied by changes in "tonus". In fresh muscle the tension drops slightly below the previous level corresponding to the positive after-potential observed under the same conditions. This phenomenon is significant because it indicates the presence of a tonic contraction in "resting" muscle. After treatment with an excess of calcium ions the responses are followed by oscillatory changes in tension.

The tonus changes and the local potentials are probably manifestations of a more fundamental process, a fluctuation in resting metabolism. The mechanical changes are weak and hardly play any rôle as such. Their chief interest lies in their relation to the automaticity and rhythmicity of the muscle. It may be assumed that an increase in metabolism causes a rise in tonus and a decreased surface polarization. The decrease in polarization in turn may be considered as the last link in the chain of processes leading to the discharge of an impulse.

REFERENCES

(1) BOZLER, E.   This Journal 136: 543, 1942.
(2) BOZLER, E.   This Journal 138: 273, 1943.
(3) COLE, K. S.   J. Gen. Physiol. 25: 29, 1941.
(4) MEEK, W. J.   Physiol. Rev. 7: 259, 1927.

INEFFECTIVENESS OF ADRENAL CORTEX PREPARATIONS IN THE TREATMENT OF EXPERIMENTAL SHOCK IN NON-ADRENALECTOMIZED DOGS[1]

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The conclusion that the adrenal plays an important rôle in increasing the resistance of the organism to shock is largely derived from experiments made on adrenalectomized animals. The animal deprived of adrenals is extremely sensitive to minor stress procedures, and rapidly develops circulatory failure unless cortical extract or certain adrenal steroids are promptly administered. Whether the circulatory failure of adrenalectomized animals and shock in intact animals are due to the same functional deficiency remains to be proven. The assumption that because cortical extract will raise the resistance of the adrenalectomized animal to that of the normal, it will also prove beneficial in the treatment of shock, presupposes that the glands of the shocked intact animal are no longer able to secrete sufficient adrenal hormones to meet body requirements. At present such an assumption lacks definite experimental proof.

The available evidence concerning the therapeutic value of adrenal cortical hormones in shocked intact animals is highly conflicting. For example, Heuer and Andrus (1) found that when cortical extract was added to a plasma transfusion, the shock which follows the injection of the contents of a closed intestinal loop could be prevented. Likewise, Wohl, Burns and Pfeiffer (2) observed a beneficial effect of cortical extract, when given with infusions of physiological salt solution, in cases of high intestinal obstruction. However, Fine, Fuchs and Mark (3) reported that while desoxycorticosterone acetate (DCA) prevented the plasma volume decrease which occurs after intestinal obstruction, it had no

clear effect in preventing fatal shock. Helfrich, Cassels and Cole (4) found that cortical extract administered to dogs before shock was induced by intestinal manipulation, greatly retarded the blood pressure decline. The same workers state that in experimental shock produced by hemorrhage, cortical extract exerted a definite but slight tendency to decrease the severity of the shock, particularly when the extract was given in conjunction with glucose and electrolytes. Fine and co-workers (5), however, have recently reported inconclusive results with the use of extract following hemorrhage, and Huizenga *et al.* (6) could find no evidence of a beneficial effect of extract in carefully controlled hemorrhage experiments.

Cortical extract has been used in anaphylactic shock in guinea pigs (7) and dogs (8), with positive results in the former and negative in the latter case. Perla and co-workers (9) claimed that cortical extract plus saline protected rats against toxic doses of histamine more regularly than would the use of saline alone.

With regard to traumatic shock, Weil, Rose and Browne (10) found a marked reduction in the mortality of rabbits subjected to visceral manipulation when cortical extract was given, but no such effect was observed with DCA. Likewise, Selye and co-workers (11) found cortical extract and certain adrenal steroids to be effective after intestinal trauma in rats, but DCA was actually harmful. Noble and Collip (12) found a slight increase in the resistance of rats to the generalized trauma produced by whirling the animal in a specially designed drum. Wilson and Stewart (13) found that although DCA prevented the decline in plasma electrolyte concentrations following scalds, it did not prevent fatal shock. However, Katz and his associates report that DCA is highly effective in preventing shock in dogs subjected to venous occlusion (14). Cortical extract, while less potent in this respect, definitely showed a positive effect (15). Treatment of the shocked human patient with extract and adrenal steroids has not, in general, given clear cut results and the efficacy of such treatment is in dispute (9, 16–22).

Evidence for the therapeutic value of adrenal hormones would seem to depend on the selection of a shock inducing procedure which could be standardized, and would yield a shock condition which satisfied three basic requirements: a. The shock should terminate in death in nearly all untreated control animals, in an interval of some 4–24 hours. On the other hand, the survival period should be long enough to allow injected cortical extract or steroids sufficient time to exert full effect. b. The trauma administered should not result in such mutilation as to necessitate the animal being kept under anesthesia throughout the experiment. c. The shock involved should be reversible, and hence responsive to therapeutic measures. We have studied three shock inducing procedures which seem to satisfy these criteria for testing adrenal cortical extract or steroid therapy.

I. *Shock following release of leg tourniquets.* The release of tight tourniquets applied to both hind legs of a dog, after a 5 hour period of constriction, is followed by fatal shock in 96 per cent of untreated control dogs (23).[4] The initiating

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[4] In a previous tourniquet paper, This Journal **138**: 156, 1942, the diameter of tubing employed is erroneously given as 120 mms. This should be 12. mms.

factor seems to be the rapid and extensive leaking of plasma through the capillaries into the tissues of the injured legs. In the first hour after tourniquet release, and before clinical signs of shock are apparent, there is a 40 per cent rise in hematocrit and hemoglobin levels, and a proportional decrease in plasma volume as measured by T-1824 dye technique (24). Shock, as evidenced by a mean blood pressure level of 75–80 mm. Hg, a decline in skin temperature, pallid mucous membranes and gums, and a lessening of the need for nembutal anesthesia, follows within 3–4 hours. The survival periods of control animals subjected to constriction of both hind legs for 5 hours ranged from 3–27 hours, the average being 8 hours.

The extract used in the experiments was a concentrate prepared in this laboratory (1 cc. = 120 grams fresh adrenal tissue) with activity such that 1–2 cc. daily was sufficient to maintain a 10 kgm. adrenalectomized dog in good health and vigor with normal serum electrolytes, blood glucose and blood pressure.

It was recognized that single intravenous injections of cortical extract into an animal which was losing its plasma volume so rapidly into the injured legs, perhaps could not provide a blood hormone level constant or high enough to restore the damaged vascular bed. For example, the administration of large amounts of physiological salt solution by a single injection is followed by immediate swelling of the legs, with little discernible interruption of the rise in hematocrit level or amelioration of the shock symptoms (23). Even when a total of 50 cc. of extract was given in small doses, intravenously or intra-arterially, over a period of hours, it did not retard the development of shock, reduce the hemoconcentration, or prevent death.

The injection technique was therefore modified so that a constant source of extract was available to the animal after release of the leg tourniquets. A 20 gauge needle was connected to a sterile reservoir maintained at constant temperature, and immediately upon release of the constrictions 280 cc. of cortical extract allowed to drip into the jugular vein at a rate slightly greater than 1 cc. a minute. There was no evidence that the cortical extract so given modified the degree of hemoconcentration or the usual sequence of events leading to fatal shock (table 1, dog 1).

Death following release of leg tourniquets can be prevented by wrapping the injured legs in tight bandages, covered with adhesive tape, immediately following tourniquet release (23). This procedure reduces the volume of plasma lost into the legs, and hence decreases the hemoconcentration and decline in plasma volume. If these bandages are allowed to remain for a sufficient length of time, the capillaries of the legs recover and circulation is restored. The bandages can then be removed without a marked increase in hemoconcentration and development of fatal shock. The critical period for rehabilitation of the capillaries would seem to lie between 18 and 24 hours. At least, when the bandages were removed at the end of 24 hours, 2 of 4 dogs showed a subsequent extreme hemoconcentration and died in shock, and the other 2 exhibited but moderate blood concentration, followed by a slow hemodilution, and eventually recovered. An attempt to remove the bandages at the end of 18 hours in 3 additional dogs was followed by fatal shock in every case.

The bandaging technique seemed ideally suited to a crucial test of the therapeutic value of cortical extract, since it lengthened the time over which administered hormones could exert an influence.   The patency of the circulation in the bandaged limbs was shown by injecting the dye T-1824 into the saphenous vein, and recovering it within a few seconds from the jugular.   A total of 50

TABLE 1

Non-effectiveness of cortical extract and of desoxycorticosterone acetate in the treatment of shock following release of leg tourniquets and following muscle trauma in intact dogs

TIME	BLOOD PRESSURE	PULSE	HEMATO-CRIT	HEMO-GLOBIN	REMARKS
Dog 1. 10.2 kgm. Tourniquet shock, given 280 cc. cortical extract					
	mm. Hg	*per minute*	*per cent*	*grams per cent*	
10:15 a.m.	108	130	42.1	15.3	Tourniquet applied to both hind legs
3:15 p.m.	110	166	46.0	16.3	Tourniquets released. Started infusion of cortical extract by intravenous drip
7:15 p.m.	75	180	71.2	29.3	Infusion completed. Dog died at 11 p.m.
Dog 2. 13.6 kgm. Tourniquet shock, 18 hour leg bandaging, given 50 cc. cortical extract					
8:00 p.m.	108	104	32.8	12.6	Tourniquets released after 5 hr. constriction period, tight bandages applied. Given 10 cc. cortical extract intravenously at 9 p.m., 11 p.m., 8 a.m., 11 a.m. and at 2 p.m. when bandages removed
6:30 p.m.	67	190	66.7	19.8	Dog died at 7 p.m.
Dog 3. 6.1 kgm. Tourniquet shock, given 5 transfusions of 3.1 cc. plasma concentrate each, and 5 injections of 6.2 cc. cortical extract each					
3:00 p.m.	100	144	37.9	13.8	Tourniquets released, first transfusion
4:00 p.m.	62	80	59.1	21.4	Second transfusion
5:00 p.m.	68	128	56.7	22.3	Third transfusion
7:00 p.m.	63	184	60.0	22.3	Fourth transfusion
10:00 p.m.	62	172	63.6	23.4	Fifth transfusion. Dog died at 11 p.m.
Dog 4. 6.2 kgm. Muscle trauma shock, given 70 mgm. DCA fore-treatment and 75 cc. cortical extract					
9:45 a.m.	103	120	44.0	17.3	Trauma of 1000 blows completed at 10:15 a.m. Extract in 10 cc. doses given half-hourly
4:00 p.m.	32	208	38.8	17.5	Dog died at 4:25 p.m.

cc. of extract, in divided doses, was then given to each of 3 dogs whose hind legs were tightly encased in bandages.   When the bandages were removed at the end of 18 hours, the legs swelled, the blood concentrated, and death from shock ensued (table 1, dog 2).   It would seem that the injected extract had neither prevented shock nor hastened repair of the capillary bed in the injured legs.

The steroid DCA[5] was also used on a series of dogs with legs bandaged for 18 hours after release of tourniquets. Since this hormone was in oil, repeated injections were made intramuscularly over the 24 hours preceding the experiment as a prophylactic treatment. The results were similar to those where cortical extract was employed, since as much as 50 mgm. DCA did not prevent shock or reduce the hemoconcentration when the bandages were removed.

The possibility remained that cortical extract might be effective against this relatively severe type of shock only if given in conjunction with a plasma transfusion. A transfusion of 25 cc. of dog plasma per kilogram body weight could prevent death after release of the leg tourniquets, only when given in a certain time sequence (23). A single transfusion, whether given immediately upon release of the tourniquets or later when the animal was already in shock, prevented death in only a few cases. When, however, the same volume of plasma was divided into 5 equal portions, and injected at the time the tourniquets were released, and at the end of the 1st, 2nd, 4th and 7th hours thereafter, fatal shock was prevented in all cases tested.

The relative ineffectiveness of the single transfusion might be explained by assuming that the rapid expansion of the circulating plasma volume had increased the rate of plasma transfer through the damaged capillaries, so that little permanent benefit would result. To test this point, a plasma protein concentrate was made by dialyzing freshly prepared dog plasma until free of salts, concentrating it to a third its original volume under reduced pressure at low temperature, and adding NaCl to 0.9 per cent. A single injection of 5 cc. per kgm. body weight of this concentrate could not prevent death in 4 dogs following release of tourniquets. When the 5 cc. per kgm. was divided into 5 injections of 1 cc. per kgm. each, and given intermittently over a 7 hour period, fatal shock was prevented in 4 of 5 dogs. It seems obvious that the difference in the efficacy of the two transfusion procedures was not merely a reflection of the volume administered, but depended on the time sequence with which they were given.

The amount of plasma concentrate, administered in 5 equal doses, was then step-wise reduced until fatal shock could no longer be prevented. For instance, a total of 3.5 cc. per kgm. body weight allowed successful recovery of 2 of 3 dogs. A dose of 2.5 cc. per kgm. did not prevent death in 3 additional animals. Thirty-five cubic centimeters of the cortical extract was then given in conjunction with a transfusion of 2.5 cc. kgm. body weight in 2 dogs. Both showed hemoconcentration changes similar to those of the controls, and died in the usual interval (table 1, dog 3). Likewise prophylactic treatment with 70 mgm. DCA did not allow recovery when the fore treatment was followed with a transfusion of 2.5 cc. plasma protein concentrate per kgm. body weight in 2 more dogs.

It seems clear that in no case, in a series of 14 dogs subjected to a 5 hour constriction of both hind legs, did either cortical extract or DCA reduce the plasma loss into the injured tissues, hasten the repair of the leg capillaries, allow a

greater retention of administered plasma, increase the efficacy of a transfusion, or prevent the onset of fatal circulatory collapse.

II. *Shock following leg trauma.* When all faces of the thigh muscles of both hind legs of the etherized dog were bruised by 800–1600 rapid blows delivered with a light rawhide mallet, according to the technique of Best and Solandt (25) as modified by Gregersen and his associates (26), fatal shock followed in 33 of 37 untreated control animals (27, 28). The survival periods ranged from 2 to 8 hours, with an average of 4 hours. Shock induced by this procedure can be prevented by administering spinal anesthesia just previous to trauma, or by multiple injections of 4 per cent procaine into the skin and muscles of the area to be traumatized (27). Significant changes in hematocrit, hemoglobin, or serum protein levels do not occur in this type of shock, although the plasma volume is reduced by an average of 32 per cent.

Cortical extract (35–70 cc.) and DCA (40–70 mgm.) were given to 10 dogs subjected to muscle trauma. All animals died in shock, without significantly prolonged survival periods. Thus cortical extract and the synthetic steriod DCA were without discernible effect in preventing shock produced by trauma to muscle masses of the hind legs.

III. *Shock following venous occlusion.* The technique of Perlow and co-workers (29) was followed as closely as possible in this experiment. Using ether anesthesia, and aseptic precautions, the internal and common iliac veins of the left hind leg were exposed by retroperitoneal approach, and ligated. Twelve to 15 cc. of a freshly autoclaved, 1:20 suspension of lamp black in physiological saline was then injected into the external iliac vein, to block collateral circulation through minute vessels. It is important that the lamp black suspension be thoroughly shaken immediately before transferring to an oiled syringe, and also before injection. A ligature was placed distal to the injection site to prevent back flow of the injected suspension. The incision was then closed, ether discontinued, and the animal placed in its cage without access to water. A continuous and marked swelling of the leg followed. The blood pressure fell progressively, the pulse rate rose, and fatal shock followed in 11 of 12 control animals. The survival periods ranged from 5 to 17 hours, the average being 9 hours. The hematocrit level rose slowly but progressively from an average initial value of 50.3 per cent to 56.2 per cent, the hemoglobin level from 17.4 to 19.5 grams per cent, and the serum protein concentration from 6.05 to 6.29 grams per cent. The plasma volume, determined by the T-1824 technique (24) on 2 dogs of this series, showed a fall from an initial value of 52.5 cc. per kgm. to 25.8 cc. per kgm. body weight by the time severe shock was present. Since this reduction is much greater in degree than might be expected from the rise in hematocrit, hemoglobin and serum protein levels, it was assumed that whole blood as well as plasma was lost into the tissues of the injured leg. This conclusion was also reached by Perlow and associates on the basis of microscopic observation of the leg tissues (29).

Two more dogs were then prepared in the usual manner, but they were allowed water *ad libitum* upon recovery from the ether anesthesia. Both drank freely,

taking 500 and 550 cc. respectively, during the course of the experiment. Hemodilution followed in both cases, but there was no apparent reduction in the leg swelling, and both animals died in shock in the usual time interval.

A series of 15 more dogs was treated with DCA. Twelve of these were given dosage levels in the range reported highly effective by Katz and co-workers (14), i.e., 30–40 mgm. were given subcutaneously in divided doses at 24, 18 and 12 hours before the experiment, and 10–30 mgm. more were given intramuscularly after the operation. Six animals of the series were allowed to take water, six were not. Of those not allowed water, 5 died in shock in the usual interval. Hemoconcentration was not significantly different from that of control animals, but the serum protein levels showed a slight decline instead of the usual slight increase. All 6 animals given water drank copious amounts (the average being 1130 cc.) and, like the controls similarly treated, showed hemodilution. The serum protein levels declined from an average of 5.59 to 4.14 grams per cent. All animals died in shock, although the survival period of one was prolonged to 47 hours.

Since the possibility remained that insufficient hormone had been given, 3 more dogs were given 70 mgm. DCA as a prophylactic treatment over the 48 hours preceding the experiment, followed by an additional 40 mgm. given over the first 8 hours following the operation, to make a total of 110 mgm. per animal. Again all dogs died in shock in the usual interval. Hence in the complete series of 15 animals given DCA treatment, but one dog recovered, which, when compared with survival of the control series, indicates that DCA did not protect against the fatal shock which follows venous occlusion of the hind limb of the dog, nor was the survival period definitely prolonged.

DISCUSSION. A study of circulatory failure in adrenalectomized dogs led to the suggestion that cortical extract might prove of value in the treatment of human shock (30). More recent study of the circulatory changes found in adrenal insufficiency, and of the factors involved in producing the extreme sensitivity of these animals to stress procedures, has thrown doubt upon the premise that this "shock-like" circulatory collapse has a similar etiology to that of shock seen in the animal with intact glands. The decreased resistance of the adrenalectomized animal to circulatory stress is apparently related to the loss of carbohydrate-active adrenal hormones, and is a manifestation of a break-down in the normal intermediary metabolism of carbohydrate in the tissues of the body (31, 32). This fundamental metabolic defect is reflected in a reduced capacity of the smooth muscles of the arterioles to maintain a prolonged contraction, and hence make sustained capacity adjustments at the vascular periphery. The capillaries likewise appear affected, becoming atonic and increasingly permeable (33). Whether this effect is also due to the lowered ability of the organism to properly metabolize carbohydrate is not yet fully established (33, 34). The heart is probably also affected (35). The ability of cortical extract and the carbohydrate active steroids to increase the resistance of the adrenalectomized animal to circulatory stress procedures, and to revive the animal from circulatory collapse, appears to depend upon the restoration of the normal metabolic

processes in the tissues concerned. So far as is known there is no evidence that a derangement of carbohydrate metabolism is concerned in the shocked intact animal whose adrenal glands are presumably functional.

The shock resulting from release of tourniquets and that following venous occlusion of the leg is due chiefly, if not entirely, to local loss of blood fluid into the limbs with resulting reduction of circulating volume. In tourniquet shock the fluid lost is chiefly plasma whereas in venous occlusion much whole blood also goes out of the circulation.

Shock following muscle trauma to the hind legs is due to two factors: 1, local fluid loss at the site of injury, and 2, nervous factors initiated by a flow of nociceptive stimuli from the traumatized area. Hence in these experiments cortical hormones appear ineffective in two types of shock, that due primarily to local fluid loss and injury of capillaries, and that due to a combination of this factor with nervous influences.

SUMMARY

1. The therapeutic value of adrenal cortical extract and of the synthetic adrenal hormone DCA, has been tested in intact dogs, shocked by three different procedures: 1, constriction of both hind legs with tourniquets; 2, trauma to muscle masses of both hind legs, and 3, venous occlusion of one hind limb. Neither extract or DCA proved beneficial in the treatment of shock resulting from these procedures.

2. An explanation is offered for the discrepancy between these negative results obtained following use of adrenal cortical extract and DCA on shocked intact dogs, and the positive results obtained with these hormones on adrenalectomized animals. This explanation is based upon the difference in etiology of circulatory failure in the two types of animals.

REFERENCES

(1) HEUER, G. J. AND W. O. ANDRUS. Ann. Surg. **100**: 734, 1934.
(2) WOHL, M. G., J. C. BURNS AND G. PFEIFFER. Proc. Soc. Exper. Biol. and Med. **36**: 549, 1937.
(3) FINE, J., F. FUCHS AND J. MARK. Proc. Soc. Exper. Biol. and Med. **43**: 514, 1940.
(4) HELFRICH, L. S., W. H. CASSELS AND W. H. COLE. Am. J. Surg. **55**: 410, 1942.
(5) FINE, J., J. FISCHMANN AND H. A. FRANK. Surgery **12**: 1, 1942.
(6) HUIZENGA, K. A., B. L. BROFMAN AND C. J. WIGGERS. Proc. Soc. Exper. Biol. and Med. **52**: 77, 1943.
(7) WOLFRAM, J. AND R. L. ZWEMER. J. Exper. Med. **61**: 9, 1935.
(8) DRAGSTEDT, C. A., M. A. MILLS AND F. B. MEAD. J. Pharmacol. and Exper. Therap. **59**: 359, 1937.
(9) PERLA, D., D. G. FRIEDMAN, M. SANDBERG AND S. S. GREENBERG. Proc. Soc. Exper. Biol. and Med. **43**: 397, 1940.
(10) WEIL, P., G. B. ROSE AND J. S. L. BROWNE. Canad. M. A. J. **43**: 8, 1940.
(11) SELYE, H., C. DOSNE, L. BASSETT AND J. WHITTAKER. Canad. M. A. J. **43**: 1, 1940.
(12) NOBLE, R. L. AND J. B. COLLIP. Quart. J. Exper. Physiol. **31**: 201, 1942.
(13) WILSON, W. C. AND C. P. STEWART. Edinburgh Med. J. **46**: 153, 1939.
(14) KATZ, L. N., S. T. KILLIAN, R. ASHER AND S. PERLOW. This Journal **137**: 79, 1942.
(15) SHLESER, I. H. AND R. ASHER. This Journal **138**: 1, 1942.

(16) WILSON, W. C., A. R. MACGREGOR AND C. P. STEWART. Brit. J. Surg. **25**: 826, 1938.

(17) SCUDDER, J. Shock. J. B. Lippincott Co., 1940.

(18) IVORY, H. S. Military Surg. **87**: 423, 1940.

(19) BESSER, E. L. Arch. Surg. **43**: 249, 1941.

(20) RHOADS, J. E., W. A. WOLFF AND W. E. LEE. Ann. Surg. **113**: 955, 1941.

(21) KEATING, F. R., M. H. POWER AND E. H. RYNEARSON. Proc. Staff Meet., Mayo Clinic **17**: 59, 1942; J. Clin. Endocrinology **2**: 53, 1942.

(22) KOSTER, H. AND L. P. KASMAN. Arch. Surg. **45**: 272, 1942.

(23) SWINGLE, W. W., J. W. REMINGTON, W. KLEINBERG, V. A. DRILL AND W. J. EVERSOLE. This Journal **138**: 156, 1942.

(24) GREGERSEN, M. I. AND J. D. STEWART. This Journal **125**: 142, 1939.

(25) BEST, C. H. AND D. Y. SOLANDT. Canad. M. A. J. **43**: 206, 1940.

(26) GREGERSEN, M. I. Unpublished work.

(27) EVERSOLE, W. J., W. KLEINBERG, R. R. OVERMAN, J. W. REMINGTON AND W. W. SWINGLE. Unpublished work.

(28) SWINGLE, W. W., W. KLEINBERG, R. R. OVERMAN, J. W. REMINGTON AND W. J. EVERSOLE. Unpublished work.

(29) PERLOW, S., S. T. KILLIAN, R. ASHER AND L. N. KATZ. This Journal **134**: 755, 1941.

(30) SWINGLE, W. W., J. J. PFIFFNER, H. M. VARS, P. A. BOTT AND W. M. PARKINS. Science **77**: 58, 1933.

(31) SWINGLE, W. W., J. W. REMINGTON, V. A. DRILL AND W. KLEINBERG. This Journal **136**: 567, 1942.

(32) REMINGTON, J. W., V. A. DRILL, W. KLEINBERG AND W. W. SWINGLE. Endocrinology **30**: 692, 1942.

(33) MENKIN, V. This Journal **129**: 691, 1940; Proc. Soc. Exper. Biol. and Med. **51**: 39, 1942.

(34) FREED, S. C. AND E. LINDER. This Journal **134**: 258, 1941.

(35) FOWLER, J. L. A. AND R. CLEGHORN. This Journal **137**: 371, 1942.

THE EFFECTS OF DIRECT CHEMICAL AND ELECTRICAL STIMULATION OF THE RESPIRATORY CENTER IN THE CAT[1]

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Many attempts have been made to localize the respiratory center by progressive sectioning or destruction of the brain stem, by electrical stimulation, by study of respiratory action potentials and by clinical observations (for bibliography, see (13)). One of the most recent of these studies has been that by Pitts, Magoun and Ranson (13); they observed marked inspiratory and expiratory responses in the cat following electrical stimulation of the brain stem using the Horsley Clarke instrument, and concluded that the inspiratory and expiratory centers each occupy about 30 cu. mm. of the medullary reticular formation overlying the region of the inferior olives. This study led to a desire to obtain further knowledge concerning the actions of chemical substances introduced directly into the medulla, and to correlate results obtained from chemical and electrical stimulation at the same points.

METHODS. Cats, 2.5 to 3.5 kilos, were anesthetized with 36 mgm. of sodium pentobarbital per kilo intraperitoneally or were decerebrated under evipal anesthesia. The supraoccipital and interparietal bones were removed and the vermis of the cerebellum was scooped out thus exposing the floor of the fourth ventricle. In some cases the tentorium was removed to facilitate exploration of the middle and upper pons. The cat's head was then fixed firmly in the Horsley-Clarke stereotaxic instrument, in the electrode holder of which was fixed a fine hollow steel needle, 0.5 mm. outside diameter, insulated except at the tip. The needle was so arranged that it could be used both for injection of chemicals and for electrical stimulation. For the latter a thyratron regulated discharge was employed, the frequency of stimulation usually being 270/second and the voltage 1.5–4.0 volts measured by a cathode ray oscillograph with the needle in the medulla; a large copper rod inserted in the rectum served as the indifferent electrode.

A calibrated screw plunger was used for injecting chemicals; the amount injected varied from 1 to 5 cu. mm., and averaged 2 cu. mm. The chemicals most frequently used were sodium bicarbonate-CO_2 buffers, acids (lactic, hydrochloric, and carbonic), potassium chloride, acetylcholine, nicotine and strychnine. Blood pressure was recorded from the femoral artery by means of a mercury manometer. Respiration was measured by a spirometer to which oxygen was continuously added; CO_2 was absorbed by a cannister of soda lime between the tracheal cannula and the spirometer.

A typical experiment was conducted as follows: first the position of the obex was carefully determined in relation to the three planes of the Horsley Clarke instrument. Then the needle, filled with the solution to be tested, was inserted into the desired point in the brain stem, the response to electrical stimulation was determined, 1 to 2 cu. mm. of the solution was injected, and the electrical response again was tried; this procedure was repeated at various spots as long as the cat remained in good condition. Since it was not feasible to locate all injection sites by serial sections in the large number of cats used (115 in all), the points have been charted in relation to the obex, the midline, and the surface of the brain stem. Since the underlying structures in the cat's brain are remarkably constant in relation to surface markings if cats of fairly uniform size are used, it was felt that the composite picture derived from a very great number of surface localizations would offset the lack of more direct and precise histological localization.

RESULTS. The effects produced by electrical responses were in all respects similar to those reported by Pitts, Magoun and Ranson. The most vigorous
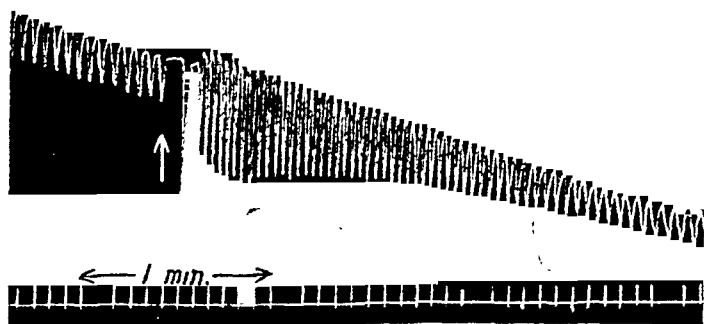
Fig. 1. Cat. Nembutal. Spirometric tracing of respiration. At the arrow, 2 cu. mm. of CO_2-bicarbonate solution (pH 7.4, pCO_2 200 mm. Hg) was injected into the medulla.

responses were elicited by stimulation in the region from 1 mm. below the obex to 3 mm. above it. Furthermore expiratory and inspiratory areas were fairly sharply defined. As a rule the former were dorsal and the latter ventral; 74 per cent of all expiratory responses were elicited by stimulation of spots 1 to 4 mm. deep, while 78 per cent of all inspiratory responses occurred following stimulation 3 to 7 mm. below the surface of the area outlined above.

While the usual effect produced by electrical stimulation was an apnea in the inspiratory, expiratory or mid position or a shifting of respiration toward inspiratory or expiratory levels, the usual effect produced by chemical stimulation was a prompt increase in depth or in both depth and rate often amounting to marked hyperpnea lasting several minutes (fig. 1). Occasionally following chemical stimuli expiratory apneas were observed and rarely inspiratory apneas were seen; in some instances the typical hyperpnea was preceded by a brief inspiratory or expiratory apnea. The effects of chemical stimuli also differed from those of electrical stimuli in that the latter repeatedly produced the same effect while the response from chemical stimulation was not fully repeatable as a rule. In a

few instances, however, three injections at the same spot led to hyperpneas of similar duration and intensity. In general the injections produced more tissue damage than the simple puncture involved in electrical stimulation. This was attested to not only by the lack of repeatability following injections, but also by the appearance of edema in histological sections and by the fact that electrical stimulation was occasionally less effective after injections than at the same spot beforehand. On the other hand as many as 80 injections totalling 230 cu. mm. of fluid were made into one cat's brain stem in addition to 90 electrical stimulations, and yet there was no outward change in respiration over a period of four hours.
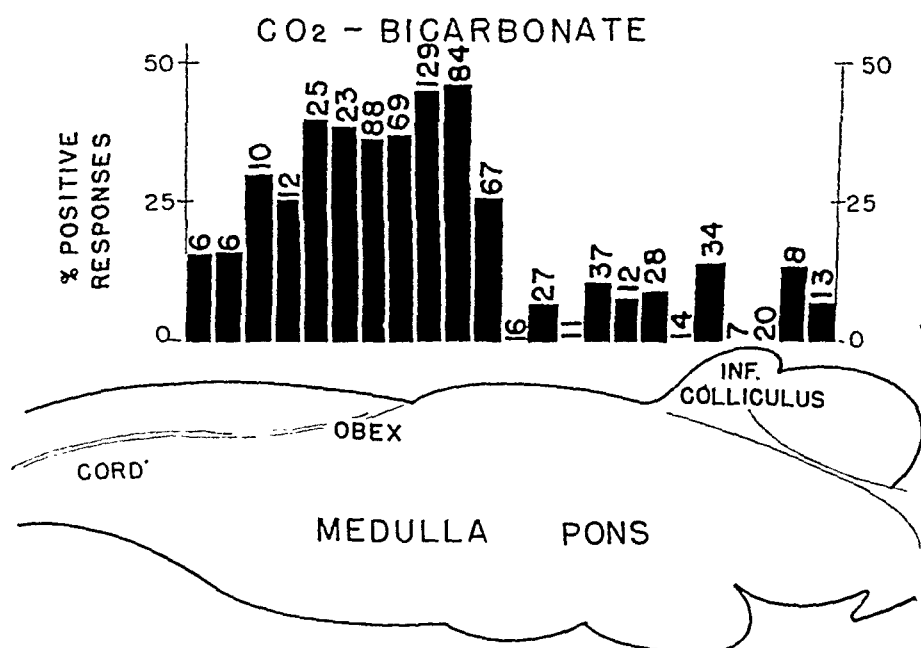
Fig. 2. Solid bars represent the per cent positive responses to CO_2-bicarbonate solutions injected into the pons, medulla and upper portion of the spinal cord. Each bar represents injections made at 1 mm. intervals into portions of the brain stem represented immediately beneath the bar. The numbers above the bars indicate the total injections performed at that level, irrespective of distance from midline or below surface of the brain stem.

The areas which yielded positive responses following chemical stimulation can be seen in figure 2. This region corresponds roughly to the respiratory center previously outlined by electrical stimulation, though the area responding to injected chemicals is slightly larger, due in all probability to a spread of the stimulants. A sufficient number of injections has not yet been made in the region of the pneumotaxic center to warrant any conclusions concerning its ability to respond to chemicals.

It is noteworthy that although the maximal effects of both electrical and chemical stimulation were obtained from the same region, at any given point close correlation was not necessarily observed. Of the spots at which chemical stimulation produced marked hyperpnea, electrical stimulation produced inspiratory

responses in 60 per cent but at 18 per cent of these points there was no response to electrical stimulation. Furthermore 14 per cent of the points which were completely negative to chemical stimulation gave inspiratory responses when stimulated electrically, and an additional 14 per cent which failed to respond to injections yielded expiratory responses following electrical stimulation (see table 1).

As shown in figure 3, one of the most effective chemical stimuli was a 1.3 per cent solution of $NaHCO_3$ buffered to pH 7.4 by CO_2 so as to have a pCO_2 of

TABLE 1

TYPE OF RESPONSE FOLLOWING INJECTION OF CO₂-NaHCO₃ SOLUTIONS	NO. OF OBSERVATIONS	TYPE OF RESPONSE FOLLOWING ELECTRICAL STIMULATION			
		Inspiratory	Expiratory	No change	Miscellaneous
		per cent	per cent	per cent	per cent
Moderate to marked hyperpnea......	83	60	7	18	15
Slight hyperpnea....................	99	40	9	30	20
Depression of respiration—Expiratory response.....................	80	20	60	9	11
No change...........................	420	14	14	55	17

In this table are included only those experiments in which responses to electrical and chemical stimulation were tested at the same points.

CO₂ - NaHCO₃   418

NaHCO₃   92

ACIDS   ■ 337

CO₂ -- NaCl | 102

AC. CHOLINE | 128

5   10   15   20   25   30   35   40   45
% OF INJECTIONS RESULTING IN RESP. STIM.

Fig. 3. Per cent of injections resulting in respiratory stimulation. This chart includes only injections into the medulla (2 mm. below to 4 mm. above the obex); however injections at all depths and at varied distances from the midline are included.

250 mm. This was effective in amounts as small as 1 cu. mm. Numerous controls such as distilled water, 0.9 per cent saline, and 2 per cent saline repeatedly gave negative results.

Of 750 injections of the CO_2-$NaHCO_3$ solution into the medulla and pons of 65 cats (from 6 mm. below to 16 mm. above the obex), 214 (29 per cent) resulted in immediate mild to vigorous stimulation of respiration (see fig. 2). Of 418 injections into the medulla alone (2 mm. below to 4 mm. above the obex) 42 per cent were positive. The latter figures include injections at all depths of the medulla and at varied distances laterally from the midline; if only the injections

in the center of this region (corresponding to the reticular formation) are considered, 80 per cent of the injections resulted in positive responses.

In addition to the responses described, injections at 80 points resulted in temporary expiratory apnea or in a decrease in rate or depth of respiration. Since in 60 per cent of the cases (table 1), these same spots responded to electrical stimulation by similar depression of breathing, it is reasonable to suppose that the CO_2-$NaHCO_3$ solution actually stimulated expiratory neurones.

It is of interest to note that a 1.3 per cent solution of $NaHCO_3$ in distilled water, without added CO_2 and having a pH of 8.0 or more, produced increase in rate and depth of breathing following 43 of 92 injections into the region of the medulla. Other bicarbonate solutions, hypotonic and hypertonic, with a pCO_2 of 100 to 300 mm. Hg and a pH of 7.4 produced similar responses. No consistent changes in blood pressure were observed.

In striking contrast to these effects, acid solutions (N/1000, N/100, N/10 hydrochloric or lactic acid) when injected 337 times in amounts of 1 to 5 cu.

TABLE 2

TYPE OF RESPONSE FOLLOWING INJECTION OF ACID SOLUTIONS	NO. OF OBSERVATIONS	TYPE OF RESPONSE FOLLOWING ELECTRICAL STIMULATION			
		Inspiratory	Expiratory	No change	Miscellaneous
		per cent	per cent	per cent	per cent
Moderate to marked hyperpnea......	2	100			
Slight hyperpnea....................	12	32	16	24	24
Depression of respiration—Expiratory response......................	78	28	28	22	22
No change............................	254	40	21	19	20

In this table are included only those experiments in which responses to electrical and chemical stimulation were tested at the same points.

mm. into the areas of the medulla shown to be most sensitive to CO_2-$NaHCO_3$ solutions, produced only minimal responses: in 8 instances barely perceptible stimulation of respiration occurred, in 4 slight hyperpnea occurred and in one, an inspiratory apnea developed. Thus acid solutions produced only 3.8 per cent positive responses compared with 42 per cent positive responses following CO_2-$NaHCO_3$ solutions injected into similar areas (see fig. 3). Furthermore in many instances, following failure of lactic acid to stimulate, injection of a CO_2-$NaHCO_3$ solution into the same spot led to definite respiratory stimulation. In addition, acids often showed a definite tendency to depress respiration even in regions in which electrical stimulation did not produce similar responses. In 78 instances, acid injections led to expiratory apnea or to a decrease in rate or depth of breathing, but at only 28 per cent of these spots did expiratory responses follow electrical stimulation and at an equal number (28 per cent) inspiratory responses followed electrical stimulation (see table 2). This differed markedly from the expiratory responses produced by injections of CO_2-$NaHCO_3$ solutions (see above).

Other acid solutions likewise failed to stimulate: saline solutions equilibrated with CO_2 at 70, 300, and 700 mm. pressure (pH 3.9–5.0) were injected 102 times with no respiratory responses.

Other substances injected were: 1.2 per cent KCl which stimulated in 12 of 57 injections (21 per cent) but frequently led to depression either initially or following transient stimulation; 1 to 5 per cent nicotine which stimulated weakly in 4 of 45 injections (9 per cent); 1 per cent strychnine which produced 2 doubtfully positive responses in 87 injections; 0.01 to 1 per cent acetylcholine, which in 128 injections, produced no clear cut instance of respiratory stimulation. Sodium cyanide, metrazol, sodium citrate, aminophylline, ether, sodium carbonate and carbonic anhydrase were injected in a few experiments, all with negative results, but none of these was investigated sufficiently to permit definite conclusions to be drawn.

DISCUSSION. Haldane's original concept of the specificity of CO_2 as the main physiological regulator of respiration has been modified several times since it was first suggested in 1908. The most widely accepted modification considers that respiration is controlled not necessarily by the level of arterial pCO_2 or even by arterial pH but by the internal acidity of the cells of the respiratory center (5). This latter hypothesis was necessary at the time to provide an explanation for the numerous instances in which respiration increased in the face of a decrease in arterial pCO_2 or of an increase in arterial pH; it suggested that though the arterial blood may become more alkaline during hemorrhage, anoxemia, etc., the cells of the respiratory center may actually become more acid and so provide the stimulus for the hyperpnea occurring under these conditions. Since 1925 however, the need for this conception has been progressively diminished by important physiological discoveries which have established beyond doubt the existence of respiratory reflexes hitherto unknown. It is now known for example that stimulation of respiration may arise reflexly from the pressure receptors in the aortic arch and carotid sinuses (due to a fall in arterial pressure as in hemorrhage) or from the chemoreceptors of the carotid and aortic bodies (due to a decrease in arterial pO_2 as in anoxemia) (9) or from proprioceptors of the muscles, tendons and joints (as in muscular exercise) (8, 2). Thus the important exceptions to Haldane's concept of the specificity of CO_2 can now be satisfactorily explained by the effects of these new reflexes and the need for conjecture no longer exists. Indeed Gesell, who formerly championed the intracellular acidity concept, recently has reversed his previous position by accepting arterial pH values as indicative of changes in pH within the cells of the respiratory center (6). Evidence against the "acid" hypothesis has been presented in this paper since bicarbonate-CO_2 buffers usually stimulated the cells of the respiratory center while acid solutions rarely stimulated and often depressed the center.

The observation that sodium bicarbonate solutions with a pH of 8.0 can stimulate respiration as vigorously as bicarbonate-CO_2 mixtures is not surprising in view of the work of Jacobs (11) who found that the CO_2 in bicarbonate solutions penetrates into the interior of cells much more rapidly than the HCO_3 ion.

However the failure of CO_2-saline solutions to stimulate in the present experiments is surprising. One explanation might be that such solutions were so acid that any tendency to stimulate by reason of CO_2 excess was offset by a tendency to depress by reason of excess acidity. Another explanation might be that high tensions of CO_2 exerted a narcotic action, but in this case transient stimulation might be expected to precede the depressant effect; furthermore CO_2-saline solutions with a pCO_2 of only 70 mm. failed to stimulate. It is probable that no simple explanation will suffice and that the situation is a complex one. It is interesting to note that the most favorable condition for stimulation of respiration by means of localized injections appears to be one in which the CO_2 molecule may penetrate to the interior of the cell while the exterior is bathed in a bicarbonate solution (i.e., CO_2-bicarbonate buffers or bicarbonate solutions).

Since all of the concepts of respiratory control that have been proposed by Haldane, Hasselbach, Winterstein and Gesell have specified either the CO_2 molecule or acidity as the specific stimulus to the respiratory center, it has become almost obligatory to interpret all new evidence in terms of CO_2 or acidity. However figure 3 shows quite clearly that the presence of bicarbonate appears to be necessary for stimulation, while the presence of a high CO_2 tension or of acidity without bicarbonate does not seem to be necessary. Therefore the possibility exists that the HCO_3 ion may be a very important factor in the control of respiration as suggested previously (1, 4). The present experiments cannot be considered as conclusive evidence because of the unusual technique employed: cerebellum, and occasionally cerebrum, removed; insertion of needle into medulla; injection of fluid tending to produce edema and lack of repeatability; sudden gush of stimulant material directly in contact with medullary cells, etc. However in favor of the validity of the results obtained by these unorthodox methods are the following facts: the responses often resembled the hyperpnea produced by CO_2 inhalation; they occurred in the region of the respiratory center as outlined by electrical stimulation; bicarbonate solutions produced a high percentage of positive responses, whereas acids (including carbonic acid) rarely produced hyperpnea; and finally bicarbonate solutions occasionally stimulated respiration when injected at points which had failed to respond to injection of acid a moment before.

The effects produced by injection of KCl (transient stimulation followed by depression or initial depression) are similar to those previously obtained upon respiration by perfusion of the brain (10) and of the carotid bodies; the same effects have also been observed following direct application of potassium to nerve cells (3, 12).

In view of the recently proposed neurohumoral concept of respiratory control (6), special attention should be drawn to the fact that acetylcholine failed to stimulate though injected 128 times directly into the regions of the respiratory center previously shown to be most sensitive to CO_2-bicarbonate buffers. Occa-

sional delayed hyperpnea was observed when high concentrations of acetylcholine were injected, but these can be attributed entirely to reflexes aroused by a fall in blood pressure since the hyperpnea did not occur following carotid sinus and aortic arch denervation or when the fall in blood pressure was prevented by atropine. Attention should also be called to the point that injection of chemicals into the fourth ventricle (7) is not equivalent to localized injections into the medulla. It is probable that systemic absorption takes place more readily than penetration through the 5 mm. of nervous tissue that separate the respiratory center from the surface of the brain stem. Nicotine, for example, often produced respiratory stimulation when dropped on the floor of fourth ventricle but this effect was usually abolished by denervation of the carotid and aortic bodies.

A final point which merits comment is the different types of respiratory response elicited by chemical and by electrical stimulation: chemical stimulation rarely produced an inspiratory apnea while electrical stimulation often produced intense and prolonged apneas; chemical stimulation usually led to increase in both rate and depth of breathing while electrical stimulation rarely did. It is doubtful if this can be explained upon the basis of different frequency or intensity of stimulation in the two cases, for varying the frequency and strength of the electrical stimulus over wide ranges failed to convert the apnea to a hyperpnea. It is conceivable that the electrical stimulus affected predominantly groups of axones all of which originated from inspiratory or expiratory areas, while the chemical stimulus may have affected only cell bodies. Another possible explanation is that the chemical substances spread along the needle tract and so reached both expiratory and inspiratory neurones simultaneously, whereas the electrical stimuli were more sharply localized. The explanation that seems most attractive is that the electrical stimulus only duplicates the result of neuronal activity while chemical stimuli act through one normal cause of that activity, i.e., by eliciting changes in chemical equilibria within the neurons of the center.

SUMMARY

The effects of direct chemical and electrical stimulation of the brain stem were compared in a series of 115 cats using a modification of the Horsley Clarke stereotaxic instrument. Injections of minute amounts (2 cu. mm.) of CO_2-bicarbonate mixtures were followed often by immediate hyperpnea, particularly when these were injected into the region of the respiratory center previously outlined by Pitts, Magoun and Ranson. Acids (carbonic, lactic, hydrochloric), injected in the same manner, rarely stimulated respiration and often led to respiratory depression. The significance of these findings in relation to the current concepts of respiratory control is discussed. The response to the injection of a number of other chemicals (sodium bicarbonate, hypertonic and hypotonic sodium chloride solutions, nicotine, strychnine and acetylcholine) was also tested.

REFERENCES

(1) COLLIP, J. B.   J. Physiol. **54**: 58, 1920.
(2) COMROE, J. H., JR. AND C. F. SCHMIDT.   This Journal **138**: 536, 1943.
(3) EULER, U. S. VON.   Skand. Arch. Physiol. **80**: 94, 1938.
(4) GESELL, R.   Proc. Soc. Exper. Biol. and Med. **20**: 345, 1922–23.
(5) GESELL, R.   Physiol. Rev. **5**: 551, 1925.
(6) GESELL, R., C. R. BRASSFIELD AND M. A. HAMILTON.   This Journal **136**: 604, 1942.
(7) GOLD, H. AND F. BROWN.   J. Pharmacol. **54**: 463, 1935.
(8) HARRISON, T. R., W. G. HARRISON, JR., J. A. CALHOUN AND J. P. MARSH.   Arch. Int. Med. **50**: 690, 1932.
(9) HEYMANS, C., J. J. BOUCKAERT AND P. REGNIERS.   Le Sinus Carotidien.   Paris, 1933.
(10) HOOKER, D. R.   This Journal **38**: 200, 1915.
(11) JACOBS, M. H.   This Journal **53**: 457, 1920.
(12) LARRABEE, M. G., F. BRINK AND D. W. BRONK.   This Journal **126**: P561, 1939.
(13) PITTS, R. F., H. W. MAGOUN AND S. W. RANSON.   This Journal **126**: 673, 1939.

THE EFFECT OF 17-HYDROXY-11-DEHYDROCORTICOSTERONE ON THE GROWTH OF YOUNG ADRENALECTOMIZED RATS

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One of the most convenient methods for the assay of the adrenal steroids and whole extracts is that employing the young adrenalectomized rat. In a previous communication from this laboratory (1) describing this method of assay, it was pointed out that the growth of the young adrenalectomized rats is not proportional to dosage and that "growth alone is not a satisfactory basis of assay." However, there can always be determined a certain minimum dose which, administered by single subcutaneous injection daily for 20 days to the 4-weeks-old adrenalectomized male rats weighing 50 to 60 grams, is sufficient to maintain life in at least 80 per cent of the rats and produce an average growth of at least 20 grams for the 20 day period. In this test the emphasis is upon survival, and the growth requirement only insures that the treated animals gain more weight than similar animals without treatment. This criterion of survival and growth of adrenalectomized rats as a measure of hormonal activity of whole extracts has been questioned.

Ingle (2), studying the weight changes for a period of 7 days in rats having an initial weight of 100 grams, observed that adrenalectomized rats treated with 11-desoxycorticosterone acetate and rats having sham operations, made substantial gains in weight during this period, whereas adrenalectomized rats treated with similar quantities of 17-hydroxy-11-dehydrocorticosterone lost weight. When normal rats of 180 grams or above were treated with massive doses of 17-hydroxy-11-dehydrocorticosterone, inhibition of growth and a definite loss of body weight were observed (3). Wells and Kendall (4) reported an inhibition of growth in young normal rats treated with 1.0 mgm. of 17-hydroxy-11-dehydrocorticosterone per day for 10 days. On the basis of these observations, Kendall has expressed the opinion (5) that the presence of this "growth-inhibiting" steroid in adrenal cortex extracts would mask the effect of other growth-promoting principles present in extracts and thus make the survival-growth test invalid as an assay method. No studies under the experimental conditions of this assay method have been reported by Kendall. Also of interest in this respect are the observations by Ingle (2) that when 17-hydroxy-11-dehydrocorticosterone was mixed with 11-desoxycorticosterone the rats gained weight and there was no tendency toward inhibition of growth until massive dosages were administered.

In an earlier publication from this laboratory (6), it was reported that, at a dosage level of 1.0 mgm. per rat per day, 17-hydroxy-11-dehydrocorticosterone and 17-hydroxy-corticosterone maintained the life and supported growth of 4-weeks-old adrenalectomized rats of the Wistar strain. Complete assays were

not run at that time because of the small quantity of pure steroids at hand. Since more of the pure substances have now been obtained, we have been able to investigate more completely the effect on the growth of the young rats. The results of these studies demonstrate that 17-hydroxy-11-dehydrocorticosterone will maintain the young 4-weeks-old adrenalectomized rats and support growth at dosage levels of $\frac{1}{8}$ to 1 mgm. per day.

EXPERIMENTAL. Two separate experiments were carried out in this study. In each experiment, male rats of the Sprague-Dawley strain weighing 50 to 60 grams were adrenalectomized at 28 days of age. The diet was Purina Dog Chow. The animals were weighed immediately before adrenalectomy and weights were then recorded at the same hour each day until the death of the animal. The first daily injection was administered after the operation, and the same dose was repeated for the next 19 days making twenty doses in all. The materials were administered in sesame oil by subcutaneous injection and the concentrations were adjusted so that the volume of oil injected was between 0.1 and 0.2 cc. All of the rats were housed in air-conditioned animal rooms which had a temperature range of 75–79°C.

In experiment 1, the 17-hydroxy-11-dehydrocorticosterone was obtained in this laboratory by crystallization from beef adrenal gland concentrates. Its identity was proved by melting point, specific rotation, analysis, crystalline form, and comparison with a known standard in the muscle contraction test (7). The rats were adrenalectomized by the procedure previously described (1). The test substance was administered at the five dosage levels of 0.25, 0.125, 0.1, 0.067, and 0.05 mgm. per rat per day. For purposes of comparison similar dosages of a sesame oil solution of whole adrenal cortex extract were also administered.

Experiment 2 was carried out by one of us (D. J. I.) independently. This was an attempt to duplicate for the 4-weeks-old rats, weighing 50–60 grams, the experimental conditions used in the previously reported study (2) on 180 gram rats. The sample of 17-hydroxy-11-dehydrocorticosterone used in this experiment was prepared by Dr. J. J. Pfiffner of Parke, Davis and Company, Detroit, Michigan and had been found by one of us (D. J. I.), in studies conducted at the University of Pennsylvania, to be ineffective for maintaining the growth of adrenalectomized male rats of 180 grams weight. Bilateral adrenalectomies were performed under ether anesthesia according to the method of Ingle and Griffith (8). Dosage levels of 1.0, 0.25 and 0.1 mgm. per rat per day were studied and the weight changes were compared to those of sham-operated rats and untreated adrenalectomized rats.

RESULTS. The results obtained by the administration of the test substances in experiment 1 are summarized in table 1. The results obtained by the administration of 17-hydroxy-11-dehydrocorticosterone in experiment 2 are summarized in figure 1. From the results of each of the two experiments it is apparent that at dosages of $\frac{1}{8}$ to 1 mgm. per rat per day, 17-hydroxy-11-dehydrocorticosterone maintained life in these adrenalectomized rats over the 20 day period of injection and permitted about the same amount of growth as effective dosages of adrenal cortex extract. The rate of growth was not as great, how-

ever, as that of the non-adrenalectomized control rats (fig. 1) which were subjected to false operations. The greatest growth response was elicited by the highest dose (1.0 mgm.) of the pure compound.

DISCUSSION. The results clearly indicate that 17-hydroxy-11-dehydrocorticosterone is capable of supporting life and growth in 4-weeks-old adrenalectomized rats under the experimental conditions of the assay test, even when amounts are administered which are greater than those occurring in physiologic doses of cortical extracts. We have also carried out preliminary experiments with 17-hydroxycorticosterone which show that this compound will also maintain life and support growth under the conditions of the assay test and possesses, likewise, an activity of 8 to 10 rat units per milligram. These effects are char-

TABLE 1

The effect of 17-hydroxy-11-dehydrocorticosterone on the growth and survival of four-weeks-old male rats

SUBSTANCE	DAILY DOSE	NUMBER OF RATS INJECTED	AVERAGE SURVIVAL	AVERAGE GROWTH	NUMBER OF RATS SURVIVING 20 DAYS	AVERAGE GROWTH OF SURVIVING RATS ON 21ST DAY	AVERAGE SURVIVAL AFTER LAST INJECTION
			days	*grams*			
17-hydroxy-11-dehydro-corticosterone	0.25 mgm.	10	23.5	30	9	31	4
	0.125 mgm.	5	22	22	5	22	2
	0.10 mgm.	9	24.5	18.5	9	18.5	4
	0.067 mgm.	13	24.0	16.0	8	21.0	8
	0.05 mgm.	13	17	12.0	2	18.0	8
Oil solution. Adrenal cortex. Extract 105–DBK–2. 1 cc. 80 gm. gland	0.2 cc. 16 gm.	10	26	27	10	27	6.0
	0.15 cc. 12 gm.	4	24	28	4	28	4.0
	0.10 cc. 8 gm.	15	26	32	12	38	8.0
	0.075 cc. 6 gm.	10	21	13	6	18	4.0
	0.05 cc. 4 gm.	9	23	23	6	30	6.0
Sesame oil controls	0.2 cc.	27	7.2	3.4	0	—	—

acteristic of active adrenal cortex extracts administered in physiologic amounts and of the normal secretion of the animals' own adrenal glands which is assumed to include 17-hydroxy-11-dehydrocorticosterone. Our results, therefore, offer evidence supporting the validity of the survival-growth method of assay as previously described (1).

Adrenal cortex extracts and pure adrenal cortical steroids influence both electrolyte and carbohydrate metabolism (9, 10). This has stimulated a great amount of study to develop tests for evaluating the physiologic activity of extracts and pure compounds with respect to these specific functions. No one of these tests, however, should be considered adequate in itself as an assay procedure for measuring the biologic activity of whole adrenal cortex extracts,

however important it might be for following the activity with respect to one function. If either carbohydrate function tests or electrolyte balance studies are used exclusively, important fractions of the extract may be overlooked. The survival-growth test is responsive to the composite activity of adrenal cortex extracts both with respect to the known compounds and the amorphous fractions. We know of no adrenal steroid fraction which has been found active by other valid methods of assay which is not active by this test. It should, indeed, be pointed out that prolongation of the survival of adrenalectomized animals and of patients having adrenal cortical insufficiency is the most important objective to be attained in the preparation of whole adrenal cortex extracts. A biological test of this property should then logically be based upon a prolongation of survival time.
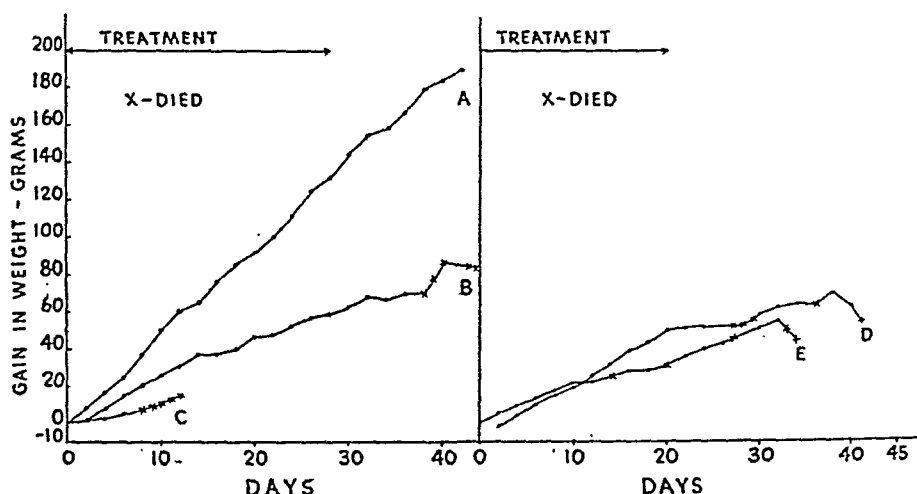
Fig. 1. Weight changes of 28 day old male, adrenalectomized rats treated with 17-hydroxy- 11-dehydrocorticosterone.

A, sham operated; B, 0.25 mgm. daily; C, sesame oil only; D, 1.0 mgm. daily; E, 0.1 mgm. daily.

The reason for the discrepancy in the results obtained earlier by one of us (D. J. I.), using rats of 180 grams weight, and the results of the present experiments, using rats of 50 to 60 grams weight, is not understood. We have insufficient data on simultaneous comparisons of the two weight groups and shall not draw conclusions at this time.

SUMMARY

The effect of 17-hydroxy-11-dehydrocorticosterone, at daily dosage levels of 0.05 to 1.0 mgm., on the survival and growth of 4-weeks-old adrenalectomized rats was determined. It was found that the compound at doses of one-eighth to one milligram is capable of maintaining the rats and supporting their growth. Whole adrenal cortex extracts which contain 17-hydroxy-11-dehydrocorticosterone and similar steroids can therefore be assayed for their complete cortical hormone activity by the method as previously described (1).

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REFERENCES

(1) CARTLAND, G. F. AND M. H. KUIZENGA. This Journal **117**: 678, 1936.
(2) INGLE, D. J. Endocrinology **27**: 297, 1940.
(3) INGLE, D. J. Endocrinology **29**: 649, 1941.
(4) WELLS, B. B. AND E. C. KENDALL. Proc. Staff Meetings of Mayo Clinic **15**: 133, 1940.
(5) KENDALL, E. C. Arch. Path. **32**: 474, 1941.
(6) KUIZENGA, M. H. AND G. F. CARTLAND. Endocrinology **24**: 526, 1939.
(7) INGLE, D. J. Fed. Proc. Am. Physiol. Soc., 1943 (in press).
(8) INGLE, D. J. AND J. G. GRIFFITH. The rat in laboratory investigation. Chap. 16. J. B. Lippincott Co., Philadelphia, 1942.
(9) INGLE, D. J. AND G. W. THORN. This Journal **132**: 670, 1941.
(10) LONG, C. N. H., B. KATZIN AND E. G. FRY. Endocrinology **26**: 309, 1940.

BACK-DIFFUSION OF UREA IN THE MAMMALIAN KIDNEY

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As recognized in 1921 by Austin, Stillman and Van Slyke (1), the rate of urea excretion is dependent on the volume flow of urine. Rehberg (11) pointed out that if the clearance of creatinine in man be taken as a measure of glomerular filtration, it would follow that urea, presumably filtered in concentration equal to that of the plasma, must be partly reabsorbed from the tubular urine. He suggested that urea might escape by diffusion from the concentrated tubular urine. Although from later work it appears that the creatinine clearance in man (but not in the dog) overestimates filtration rate, owing to some tubular secretion, Rehberg's inference of urea reabsorption has been confirmed by the use of excretable substances which give more probable estimates of filtration rate: ferrocyanide in the dog (14), creatinine in the dog (12), inulin in dog and man (13). All these studies indicate that about 40 per cent of the filtered urea is reabsorbed under conditions of moderate or high urine flows and a greater amount with low flows. Shannon pointed out, however, that a single diffusion process could not explain the phenomenon, since such a process if capable of causing the 40 per cent loss of filtered urea at high flows, would at moderately low flows lead to practical equilibration of urine and blood, contrary to experience. Hence he suggested that a minimum of two independent diffusion processes might operate, located in the proximal and distal parts of the tubules, and accompanying respectively the fixed "obligatory" reabsorption of about 90 per cent of the filtered water and the "facultative" reabsorption of variable proportions of the remaining 10 per cent of the water. In this connection "obligatory" and "facultative" are used as applied by Homer Smith (13). These conclusions, derived from the study of dogs, were extended to human kidneys by Chasis and Smith (2), who also obtained data on urea reabsorption in cases of chronic nephritis.

The equations of Austin, Stillman, and Van Slyke (1) relating urea clearance to urine volume over volume ranges above 0.5 cc. per minute, the equation of Chesley (4) for lower volume ranges, and the more comprehensive equation of Dominguez (5) covering all volume ranges, all give fairly good fits with observed data; but they are all purely empirical formulations. None of them is derived from known physico-chemical laws.

The formulation presented in this paper is based on the assumption that the decrease in urea clearance with urine volume is caused by a tubular reabsorption of urea in accordance with the quantitative laws of diffusion. The comprehensive equation thus deduced is found to fit observed data. Such fit does not, of course, prove the validity of the assumptions on which the equation is based. The simple hypothesis doubtless oversimplifies actual conditions, but it has the

advantage that it leads to a workable equation, consistent in its assumptions with the facts of renal excretion in so far as they are at present known, and capable of quantitative test by further observations.

The present formulation is offered for what assistance it may give in clarifying the steps of the excretory process. It is not recommended for clinical use as a substitute for the "maximal" and "standard clearance" formulae (9), which are simpler, and are adequate for their purposes, except for conditions of unusually small urine flows (4, 8).

Simplifying assumptions for the present analysis are:

a. Concentration of tubular urine is uniform in any cross-section of a tubule (i.e., axial flow and turbulence effects are neglected).

b. Tubule volume and permeability of the wall to urea are constant under varying conditions of flow.

c. Water reabsorption occurs at a uniform rate per unit length of tubule in each of the anatomical portions which correspond to the two assumed phases of water reabsorption, but this rate is different in the two portions.

d. The resistance to urea diffusion through the tubular wall will be treated as if it were concentrated in a single thin membrane. Actually, of course, urea returning from tubules to blood vessels must pass through tubular cells, cell boundaries and tissue space.

e. Behavior of individual tubules is sufficiently uniform that the performance of the kidney as a whole may be predicted from the analysis of a single tubule.

f. The influence of blood level on urea concentration gradient will be neglected since it is usually comparatively low and constant; when this factor is not neglected it leads to a more complicated expression differing by less than 2 per cent in predicted values.

Consider a section of tubule of radius, r and length, l_1 (fig. 1). Any arbitrary cross-section of it may be identified by the distance, l, from the origin. The volume flow of urine in cubic centimeter per minute across the plane of this cross-section will be designated by v. When the urea concentration, u at this plane is known, the rate of passage of urea along the tubule across this plane may be calculated as the product, uv. The rate, uv, at which urea crosses any plane would be the same throughout the tubule, regardless of water reabsorption, if no urea were reabsorbed; u and v would vary inversely, and the product would be constant. However, when urea is reabsorbed uv must decrease as the urine passes away from the origin. It is desired to know the rate at which urea leaves the section of tubule, given the rate, $u_0 v_0$, at which it enters the section, the rate, R, at which water is being reabsorbed per unit length of tubule and the permeability, h, of the wall to urea.

From Fick's law it is known that the rate of diffusion across a membrane is given by the product of area, permeability coefficient and concentration gradient. Expressed as a differential equation this becomes:

$$\frac{d(uv)}{dl} = -(2\pi r)hu$$

Also we have assumed that water is reabsorbed at a uniform rate per unit of tubular length, so that the volume flow across this arbitrary plane will be less than the flow at entry by the volume flow absorbed, which is $R \times l$.

$$v = v_0 - Rl$$

Eliminating l between these two equations, one obtains:

$$\frac{d(uv)}{uv} = \frac{2\pi rh}{R} \frac{dv}{v}$$
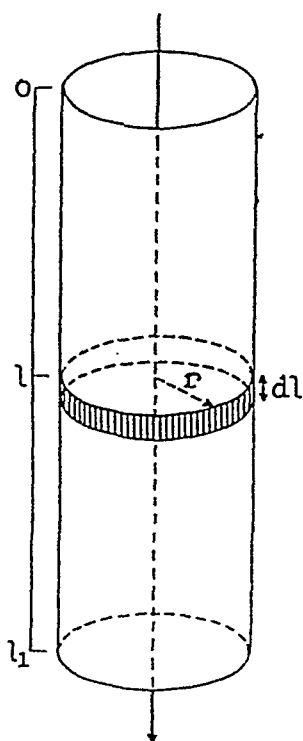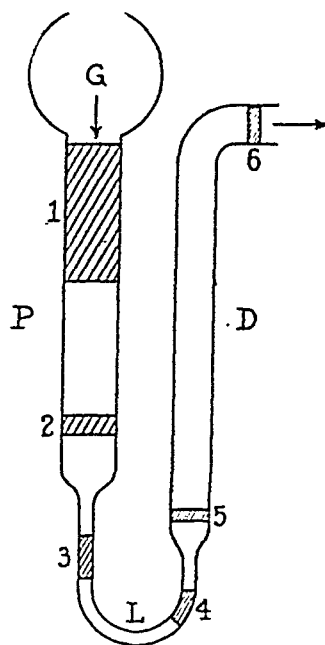
Fig. 1   Fig. 2

Fig. 1. Arbitrary uniform section of tubule.

Fig. 2. Diagram of water absorption in a single nephron (adapted from Smith (13)). A unit portion of filtrate leaving glomerulus, G, has been reduced to about one-tenth its original volume in passage through the proximal tubule, P, but with little increase in concentration of dissolved materials. In the loop, L, a further variable reabsorption of water occurs with concentration of the solutes. Volume remains constant in distal portion and subsequent course to the bladder.

Integrating:

$$\ln \frac{uv}{u_0 v_0} = \frac{2\pi rh}{R} \ln \frac{v}{v_0} = \frac{2\pi rhl}{Rl} \ln \frac{v}{v_0}$$

Let $q = 2\pi rl$ designate the total internal area of tubular wall of the section from entry to exit, and nq the area of corresponding sections in n nephrons. Also, for simplicity, let k represent the product nqh. Then the rate at which urea emerges is given by:

(1) $$u_1 v_1 = u_0 v_0 e^{\frac{-nqh}{v_1}\left(\frac{\log v_0/v_1}{v_0/v_1 - 1}\right)} = u_0 v_0 e^{-\frac{k}{v_1}\left(\frac{\log v_0/v_1}{v_0/v_1 - 1}\right)}$$

When no water is reabsorbed, $v_0 = v_1$. The factor $\dfrac{\log v_0/v_1}{v_0/v_1 - 1}$ becomes unity in this case as may be seen by setting $v_0/v_1 - 1$ equal to x, expanding $\dfrac{\log (x + 1)}{x}$ in a power series and letting x approach zero.

Thus when no water reabsorption occurs, the expression reduces to:

$$(2) \qquad\qquad u_1 v = u_0 v e^{-\frac{k}{v}}$$

Trial of these two equations indicates that the best fit of the data is obtained by a combination of the two, which is equivalent to assuming that urea reabsorption occurs successively in two phases, one associated with a concurrent water reabsorption and the second in a portion of tubule from which no water is reabsorbed. It seems reasonable to associate the first with the 90 per cent water reabsorption in the proximal tubule, and to place the second phase of urea absorption distal to the second phase of water reabsorption (fig. 2). It will be noticed that no allowance is made for urea reabsorption concurrent with the second phase of water reabsorption, since if any significant part of the urea loss is assumed to occur there, the equation fits the data less well. It seems improbable that there is no loss of urea from the tubules concurrent with the second phase of water reabsorption; it might be, however, sufficiently small to be neglected in a first approximation. It is therefore assumed that with respect to the fate of water and urea in the tubules a physiological division may be made into three successive phases. In the first both substances are reabsorbed, in the second water alone, and in the third urea alone. Tentatively, these may perhaps be identified with proximal tubule, loop and remainder of the path including the distal tubules and collecting ducts.

Multiplying equations (1) and (2) one obtains an equation which relates plasma urea clearance $Cl_u = \dfrac{uv}{p}$ to filtration rate (F):

$$(3) \qquad\qquad Cl_u = F \times e^{-\left[\frac{k_1}{v'}\left(\frac{\log F/v'}{F/v'-1}\right) + \frac{k_2}{v}\right]}$$

All variables in this equation are subject to measurement except v' which represents the volume flow of urine in the tubules at a stage between the first and second phases of water reabsorption, perhaps at the junction of the proximal tubule and the loop. Fortunately, it appears that v' is relatively constant under usual conditions in normal subjects, so that when filtration is approximately constant the first term of the exponent may be grouped into a single constant for purposes of testing the equation. Then:

$$Cl_u = F \times \phi \times e^{-\frac{k_2}{v}}$$

This factor, ϕ, which represents the fraction of filtered urea escaping reabsorption in the proximal tubules, appears to have a value of about 0.60, since 40 per cent of filtered urea appears to be absorbed in the proximal tubules. The product $F \times \phi$ gives the "maximal urea clearance" toward which observed urea clearance

appears to rise when urine flow is above the "augmentation limit" of Austin, Stillman and Van Slyke (1).

In order that the constant, k_2 may be determined from available data it is convenient to plot logarithmically the ratio $\dfrac{Cl_u}{F}$ against reciprocal of urine volume flow. This would theoretically yield a straight line since:

$$ln\,\frac{Cl_u}{F} = ln\phi - k_2\left(\frac{1}{v}\right)$$
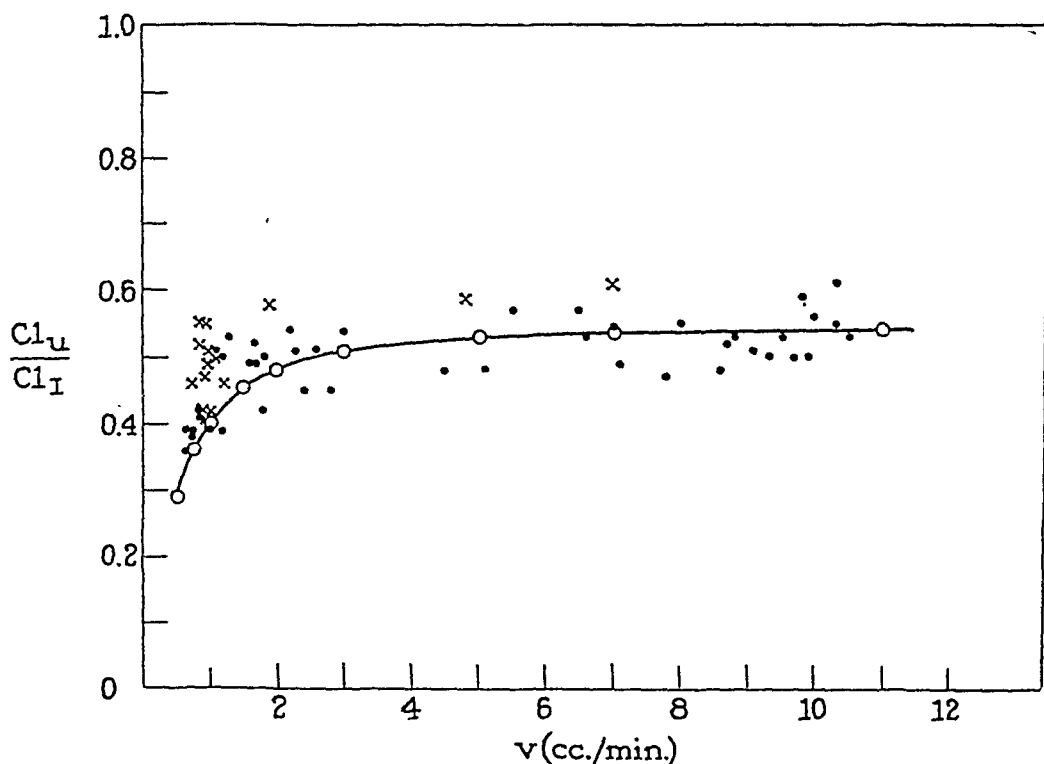
Fig. 3. Fit of theoretical equation to data from one normal human (subject T. G. (2)). Cl_u and Cl_i are plasma clearances of urea and inulin respectively. v is volume flow of urine, uncorrected for surface area. • Observed ratios on falling flows. × Observed ratios on rising flows (disregard in judging fit of the curve). ○ Theoretical points from equation $\dfrac{Cl_u}{Cl_i} = 0.57e^{-\frac{.36}{v}}$.

Aberrant clearance values due to clearance increases on rising urine flows (12) must be excluded since the simple conditions of a steady state, postulated in this analysis, are not maintained in such transitional states. Moreover, it is implied in the above simplified equation that constant filtration and constant reabsorption in the proximal tubule provide a constant v'. In extreme diuresis it appears that proximal reabsorption diminishes. This diminution appears to begin when urine flow exceeds about 10 per cent of filtration rate in normal humans (2) and about 5 per cent of filtration rate in normal dogs (12).

Normal human kidneys. A fit of the theoretical equation to data from one normal human (subject T. G. (2)) is shown in figure 3 which is a plot of the ratio

$\dfrac{\text{urea clearance}}{\text{inulin clearance}}$ against volume flow of urine. Points identified by crosses are data observed on rising urine flows; these are shown in the plot to illustrate the necessity of excluding such values.

Data covering very low urine flows (below 0.5 cc. per min.) are not included in the above paper, but are to be found elsewhere. French workers (3) over a decade ago while studying the oliguria associated with hypochloremia noted that the urine-blood ratios of urea concentration were low at extremely low flows. Chesley (4) has noted this phenomenon in oliguric normal women and found that the "standard clearance" square root correction (9) fails to make a satisfactory adjustment of what apparently is the normal behavior of urea clearance in the
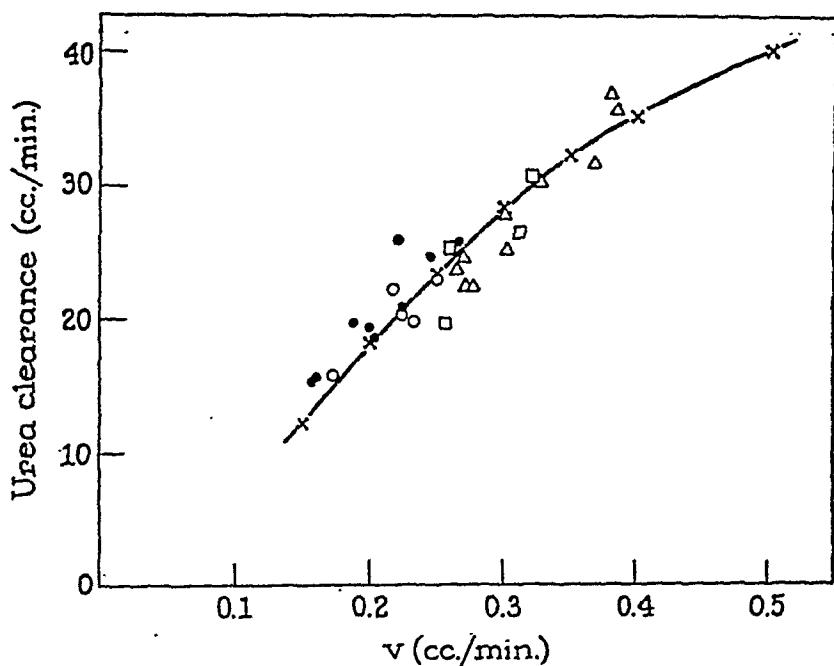
Fig. 4. Fit of theoretical equation to data from four normal oliguric subjects (4). Volume flows corrected for surface area and data from each subject multiplied by a factor to bring them on the same curve as in the original paper. • A, ○ B, △ C, ▢ D. ✕ Theoretical values from equation $Cl_u = 68e^{-\frac{.26}{v}}$.

flow range below 0.35 cc. of urine per minute. Figure 4 shows a plot of data taken from his paper (4) with the theoretical curve of the present paper fitted to it. It is to be noted that the constants (68 and 0.26)[1] have approximately the same magnitude as those in the equation fitting the data at higher flows (fig. 3). Similarly the extreme depressions of urea clearance observed by McCance in the oligurias of salt deficiency and of diabetic acidosis (8) may be explained.

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[1] If filtration at a normal rate of 120 cc/min be assumed, the ratio $\dfrac{\text{maximal clearance}}{\text{filtration}} =$

$\dfrac{68}{120} = 0.57$. The corresponding ratio in the equation of figure 3 is also 0.57.

Dominguez (5) recognized that there must be some limit to the urinary concentration of urea but obtained it by extrapolating his equation to zero flows. As volume flow approaches zero, however, the time available for diffusion becomes indefinitely prolonged; it would seem more reasonable to expect equilibration between urine and blood at this limit unless active secretion of urea by the tubules is assumed. From the diffusion equation developed in this paper the maximum urine urea concentration is to be expected at volume flows somewhere between 0.15 and 0.30 cc. per minute, a limit that cannot be accurately fixed because of uncertainty of knowledge as to the behavior of glomerular filtration in this range. Owing to the technical difficulties of obtaining extremely low urine flows, and of measuring accurately these flows and the filtration rate, it is not at present possible to make a crucial test of the prediction that the concentration ceiling of urea will be reached before urine flow is stopped and that with further decline of flow, urea will appear in not greater but lower concentration.

Normal dog kidneys. A quantitative difference in reabsorption by human and dog kidneys is to be anticipated since the surface area available for back diffusion is much less in the dog. This follows not only because the individual nephron is

TABLE 1

Number of nephrons per kidney in different species, estimated by glomerular counts (10)

SPECIES	NUMBER OF GLOMERULI PER KIDNEY
Man	700,000–1,200,000
Dog	400,000– 500,000
Cat	150,000– 225,000
Rat	20,000– 30,000
Rabbit	90,000– 250,000

somewhat smaller but more importantly because of the much smaller number of nephrons, as demonstrated by the technique of glomerular counts (table 1). The variation of number of nephrons from individual to individual is of interest since it offers one explanation of the variations in urea reabsorption.

If the permeability of the dog tubules is of the same magnitude as of human tubules, the exponent of the diffusion equation should be about one-fifth to one-tenth that found from human data. This is found to be the case, as illustrated in figure 5 taken from Shannon's data (12).

So far only that part of the equation relating to distal reabsorption has been discussed. If one assumes that at flows above those yielding a creatinine U/P ratio of about 20 (corresponding to a volume flow of about 2–4 cc. per min. in a dog) the distal reabsorption in the dog is essentially abolished, then volume flow may be taken as an index of the flow emerging from the proximal tubule. With this value, which corresponds to the quantity v' in the more general diffusion equation, it is possible to make a test against the data observed during forced diuresis in the dog (12). It may be said that theory accords with experiment in predicting an increase of $\dfrac{\text{urea clearance}}{\text{filtration}}$ ratio that approaches the limit of unity

as volume flow approaches the filtration rate. This cannot be taken as very strong support for the theory, however, since the curve followed by this increasing ratio is not accurately defined nor the assumption set forth above adequately supported.

Abnormal human kidneys. From examination of the diffusion equation (equation 3) it may be seen that three chief factors separately or in combination may be expected to alter the relation of urea clearance to volume flow in subjects with renal disease:

a. Filtration may be reduced, with the consequence that volume flows of urine which would be only moderate in a normal kidney become equivalent to marked diureses. A flow of 5 cc. per minute would, for instance, be only 4 per cent of the filtration rate if filtration were normal at 125 cc. per minute but would be 20 per cent if filtration were reduced to 25 cc. per minute.
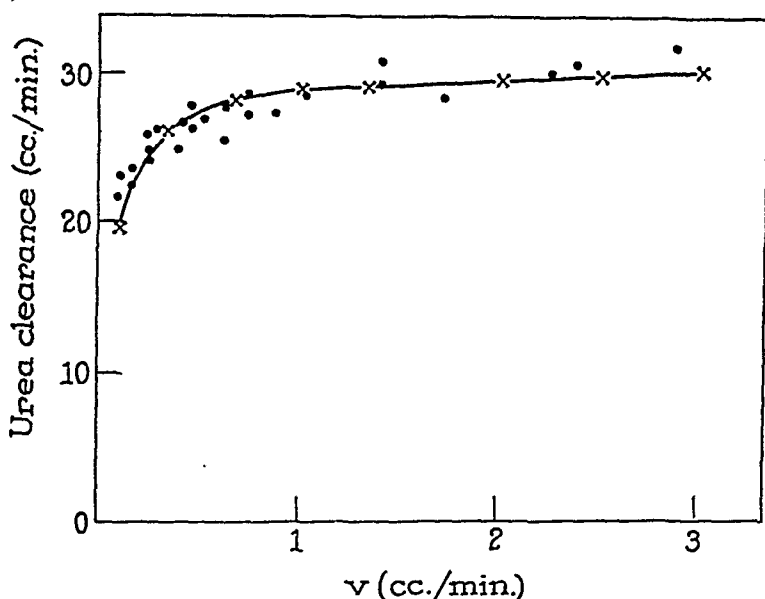
Fig. 5. Fit of theoretical equation to data from one normal dog (dog E, (12)). • Observed values. ✕ Theoretical values from equation $Cl_u = 30e^{-\frac{.042}{v}}$.

b. Tubular surface area may be reduced so that the reabsorption observed in normal kidneys at low flows would be evident only at proportionately lower flows.

c. The capacity for facultative reabsorption of water may be reduced, thus preventing the attainment of sufficiently low flows to allow appreciable reabsorption in the distal part of the tubules. In this case variations in urine flow must be largely due to variations in filtration or in proximal tubular water reabsorption or in both, so that the percentage reabsorption of filtered urea becomes dependent not only on volume flow of urine but also on the values of these two additional variables.

Sufficient data are not at present available to permit satisfactory test of this theory. The data of Chasis and Smith (2) on the relation of urea clearance to urine flow and filtration rate in nephritic subjects show general conformity to

the theoretical curves but are insufficient for present purposes. The data reported by Hayman (6) on atypical clearances following relief of prostatic obstruction show a continuous rise of clearance with volume flow. This relation would likewise be predicted if it is assumed that the tubular capacity for facultative water reabsorption has been impaired by damage due to back pressure.

Tubular permeability. The barrier to back-diffusion of urea has been treated as a thin membrane to which solutions on either side have free access. It is possible from the data to make an estimate of the order of magnitude of the permeability coefficient of this membrane, recognizing of course that the value is virtual rather than one to be identified with some particular structure. From the value it should be possible to decide whether or not the tubules present a unique barrier to back flow of urea.

In the derivation of the formula the product of permeability coefficient and total tubular surface area of the distal tubules was expressed as a single constant, k_2, for convenience in manipulation. Since trial of data from various sources (including unpublished experiments) has led to a value of about 0.3 cm.^{3}/min. for k_2, while the values of n and q_2 may be taken as approximately 2×10^6 and 10^{-2} cm.^{2}, respectively, we have

$$h_2 = \frac{k_2}{nq_2} \sim 10^{-5} \text{ cm./min.}$$

Jacobs (7) has given the permeability coefficient of ox erythrocytes for urea as 108×10^{-15} mols/μ^2/min./mol per liter. These dimensions, correctly expressed as mols/$\mu^2 \times$ min. $\times$ mol/liter, may be converted to the present dimensions of cm./min. if multiplied by the factor 10^{11}. This gives a value of about 10^{-2} cm./min. to be compared with the permeability in the order of 10^{-5} cm./min. estimated for the tubular wall. So far as comparison of such dissimilar systems is permissible, it may be inferred that the permeability of the tubular wall to urea is of a low order.

CONCLUSION

The behavior of urea clearance at varying volume flows of urine in human and dog kidneys is approximately described by assuming that urea diffuses from tubular urine to blood in two phases, related to the proximal and distal reabsorptions of water. Estimation of the permeability coefficient of the distal tubular wall for urea suggests that, as compared to ox erythrocytes, the wall has a low permeability.

REFERENCES

(1) AUSTIN, J. H., E. STILLMAN AND D. D. VAN SLYKE. J. Biol. Chem. 46: 91, 1921.
(2) CHASIS, H. AND H. W. SMITH. J. Clin. Investigation 17: 347, 1938.

(3) CHABANIER, H. AND C. LOBO-ONELL. Hypochlorémie et accidents post-opératoires. Masson et Cie, 1934.

(4) CHESLEY, L. C. J. Clin. Investigation 16: 653, 1937; 17: 119, 1938.

(5) DOMINGUEZ, R. This Journal 112: 529, 1935.
DOMINGUEZ, R. AND E. POMERENE. J. Clin. Investigation 22: 1, 1943.

(6) HAYMAN, J. M., L. P. LONGLEY AND M. BOBEY. Trans. Ass. Am. Phys. 56: 291, 1941.

(7) JACOBS, M. H. J. Cell. Comp. Physiol. 4: 161, 1933–34.

(8) McCANCE, R. A. AND E. M. WIDDOWSON. J. Physiol. 91: 222, 1937; 95: 36, 1939.

(9) MØLLER, E., J. F. McINTOSH AND D. D. VAN SLYKE. J. Clin. Investigation 6: 427, 1928.

(10) MOORE, R. A. Anat. Rec. 48: 153, 1931.

(11) REHBERG, P. B. Biochem. J. 20: 461, 1926.

(12) SHANNON, J. A. This Journal 117: 206, 1936; 122: 782, 1938.

(13) SMITH, H. The physiology of the kidney. Oxford University Press, New York, 1937.

(14) VAN SLYKE, D. D., A. HILLER AND B. F. MILLER. This Journal 113: 611, 1935.

THE CHANGES OF IMPEDANCE OF THE TURTLE'S VENTRICULAR MUSCLE DURING CONTRACTION

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The electrical impedance of striated muscle increases during contraction (Bozler, 1935; Bozler and Cole, 1935; Dubuisson, 1937). In the turtle's ventricle, on the other hand, Rapport and Ray (1927) found a decrease of impedance during systole. The present study was undertaken to determine whether or not there is a fundamental difference between striated and cardiac muscle, in this respect.

METHOD. From pithed turtles the heart was excised. The several preparations of the ventricle used will be described with the corresponding results.

The measurements of the electrical impedance were made by means of an alternating current (a.c.) bridge. The muscle was in one arm of the bridge. It was balanced against a variable calibrated capacity (mica and paper condensers) and a variable non-inductive resistance, in parallel. The balance indicator was a cathode-ray oscillograph, after capacity-coupled amplification.

The balance was never perfect, thus showing that the electrical model used is only an approximation of the muscle. As shown by the records, however, the balance was sufficient to give a clear idea of the changes of impedance in the tissue.

A beat-frequency oscillator was used for generating the alternating currents delivered to the bridge. The frequency ranged from 30 to 10,000 cycles per sec. The results were qualitatively similar with all the frequencies tested. In the majority of the experiments 200 to 1,000 cycles were employed. The intensity (m.r.s.) of the current delivered to the bridge was from 0.04 to 0.06 milliampere (ma). The current flowing through the tissue was therefore about 0.025 ma. The electrodes were chlorided silver needles or else wicks wet with Ringer solution and connected to chlorided silver plates. The usual procedure was to balance the resting tissue. The changes of impedance during activity appeared then as an unbalance. These changes could be photographed from the oscillograph. The mechanical response was photographed simultaneously from an appropriate isometric optical myograph. The electrodes on the tissue were then connected with a direct-coupled amplifier activating the oscillograph and the ventricular electrogram was registered together with the mechanogram. Using the stimulus artifact, when the responses were stimulated, and the mechanogram, as common references in the two records, the electrogram could be compared with the changes of impedance.

A change of the balance of the bridge does not indicate the direction of the corresponding change in the tissue—i.e., whether the reactance or the resistance

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[1] Fellow of the J. S. Guggenheim Memorial Foundation.

increased or decreased. Records were taken, therefore, in which the bridge was initially unbalanced in a known direction. A balancing or a further unbalancing change during activity gave then a precise indication of the change of impedance in the ventricle. Thus, if the resistance of the model was initially higher than necessary for balance, and if balance was restored during activity, an increase of resistance of the ventricle during contraction was indicated.

RESULTS. It is important in the observations of the changes of impedance due to muscular contraction to prevent movement of the electrodes and shortening of the tissue. The following procedures were found satisfactory for those purposes and yielded consistent results.

Ventricular strips were cut in some instances. One end of the strips was stretched and fixed firmly on a board by means of 4 to 6 pins. The electrodes from the bridge were applied to this portion. The other end of the strips was attached to an isometric torsion-spring myograph.

In other cases the ventricle was cannulated through the atrium. The cannula was connected to a small membrane manometer. The system was filled with Ringer solution and was closed. The contractions were thus isometric. Needle or wick electrodes were applied to the external ventricular surface, or else one such electrode was used and another was in contact with the Ringer solution inside the closed system.

The most satisfactory method was to stretch the intact empty ventricle and fix it on a board by means of 6 to 10 pins, leaving a small basal part free. This free portion was attached to a torsion-spring myograph. The alternating current electrodes were applied near some of the fixing pins, to prevent movement, and near the mechanically recording part, to obtain a satisfactory temporal correlation between the contractions recorded and the changes of impedance. This method was used in the majority of the observations.

Although all these procedures tended to minimize movement, they did not prevent it entirely. Whenever movement did occur the results were inconsistent and complicated—an increase or a decrease of impedance could appear during contraction, or else an initial increase with a later decrease, or vice versa. The results, on the other hand, were consistent and relatively simple when no movement was seen at and between the leads. The description will deal only with these observations.

Typical changes of impedance associated with a response are illustrated in figure 1. In A the resting balance is disturbed reversibly. The beginning of the change coincides approximately with that of the mechanogram. The two curves are parallel at first but the impedance change outlasts the contraction. A comparison of A with C shows that there is no parallelism between the impedogram and the electrogram. At the end of the electrical response the impedance is still far from the resting value and only returns to that value later.

As explained under Method, the direction and characteristics of the changes of impedance were studied by recording with a known unbalance. If the resistance in the electrical model in the known arm of the bridge was too low, or if the capacity was set lower or higher than the proper value for balance at rest,

the unbalance was exaggerated during contraction. If the resting unbalance was obtained by increased resistance, however, then a relative balance appeared during the response (fig. 1B). It may be inferred that the impedance of the ventricle increases during its activity and that this increase is due largely to an increase of resistance, with the frequencies of alternating current used.

This inference was supported by measurements of the settings of the bridge which gave the best balance at the peaks of a series of contractions, and by com-
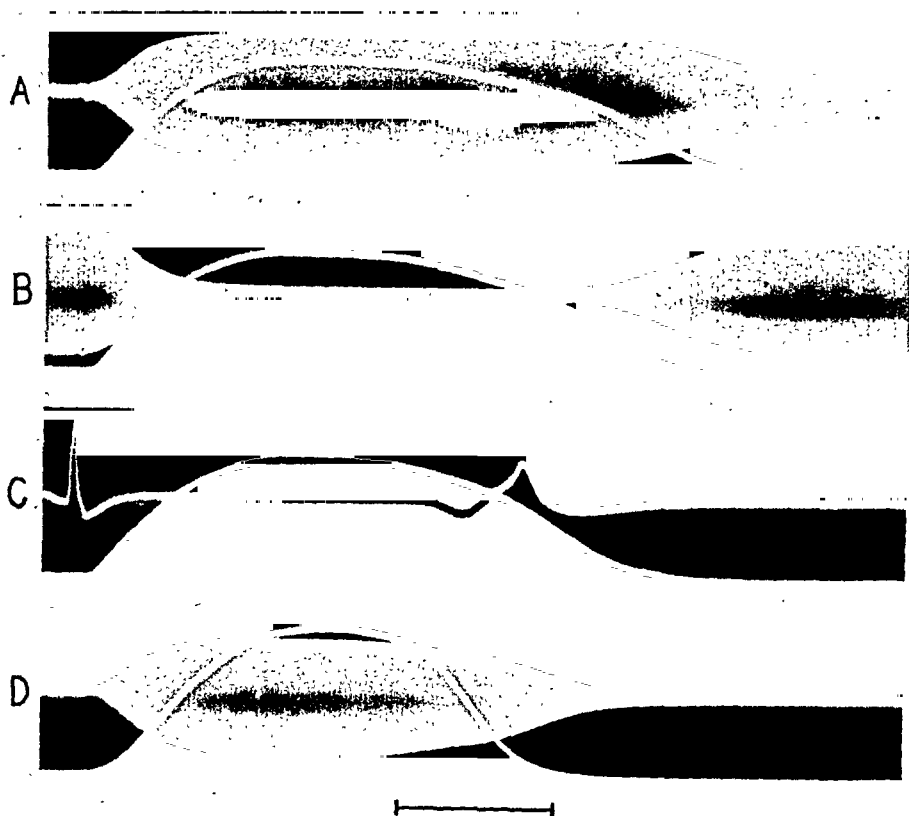
Fig. 1. Typical changes of impedance during contraction. Electrodes on two uninjured points in the ventricle. In this and other figures the lower tracing is the isometric mechanogram; the time calibration corresponds to 1 sec. The frequency of the alternating current was 500 cycles per sec.

A. Bridge balanced before stimulation. Settings of the known arm: resistance, 3130 ohms; capacitance, 0.0112μF. B. Bridge unbalanced by increasing the resistance to 3730 ohms. C. The upper tracing is the electrogram recorded from the same electrodes used for the records of impedance in A and B. D. As in A, but after warming the heart.

parison of these values with those corresponding to the resting balance. Thus, in a typical observation, with an a.c. of 0.04 m.a. and 500 cycles, the resting balance values were 3130 ohms and 0.011 μF. These values changed to 3480 ohms and 0.008 μF for balance at the peak of contraction. The impedance thus changed from 3120 to 3470 ohms.

As a rule the peak of the change of impedance coincided approximately with that of the contraction (fig. 1). Not infrequently, however, the maximum of the variation of impedance was later or, more rarely, earlier than that of the

mechanical response. It is difficult to rule out the possibility that an artifact due to movement may have determined the pattern of these atypical records. Impedograms with two peaks, early and late respectively, with reference to the maximum of contraction, were only seen when there was obvious movement of the tissue.

The duration of the impedogram varied with that of the mechanogram. Thus, when warming of the ventricle resulted in a brief contraction the variation of impedance was correspondingly brief (cf. fig. 1A and D).
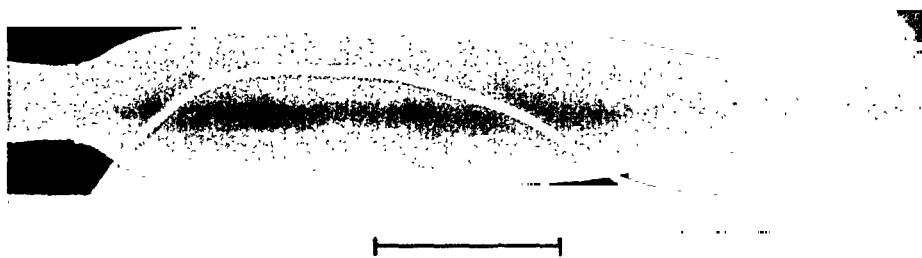
Fig. 2. As in figure 1A. Last response in a series of six, at the rate of 1 per 3.5 sec.
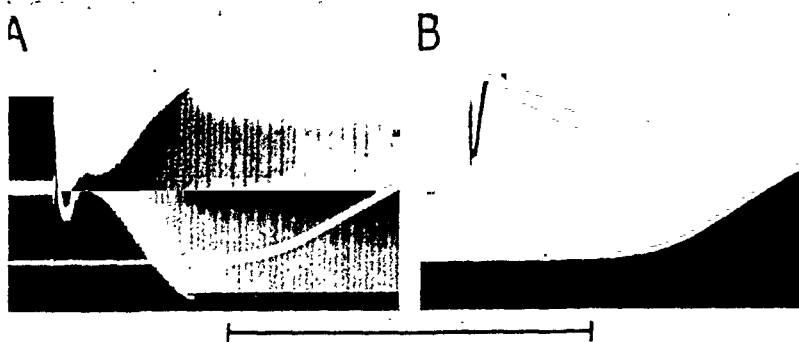
Fig. 3. Changes of impedance recorded from one electrode on intact ventricle, which was also the stimulating cathode, to another on a crushed region. Both these electrodes were at some distance from the portion of the ventricle which recorded mechanically, hence the long latency of the contraction. Only the beginning of the responses is reproduced.
A. Impedogram with about 4 times higher amplification than in figure 1. B. Electrogram recorded from the same electrodes.

Since the change of impedance outlasted the development of tension, it was possible to elicit a further response before an earlier one had fully subsided. A typical observation is illustrated in figure 2, which reproduces the 6th response in a series at the rate of 1 per 3.5 sec. The maximum variation of impedance was not greater than that corresponding to the 1st response of the series, but the subsidence of the change was slower than with single stimuli (cf. fig. 1A and fig. 2).

In order to determine whether there is any variation of impedance coincident with the beginning of the development of the electrical response, records were taken with high amplification and with one of the electrodes serving both as a stimulating cathode and as a connection to the bridge. As shown in figure 3 the electrical response began before the change of impedance.

DISCUSSION. As pointed out in the section on Method, the model of the tissue used—resistance and capacity in parallel—is quite imperfect. A perfect model would result in a perfect balance, which was not obtained here. Furthermore, with a perfect model, balance for a given condition of the tissue should not be modified by changes of the frequency and intensity of the alternating current. Such changes led invariably to unbalance in these observations.

The finding of an accurate model would involve more elaborate experiments than were carried on here (see Bishop, 1929; Cole, 1940). Notwithstanding the imperfections mentioned, the model was used because it is simple and because the measurements of the changes of impedance are accurate—i.e., if the impedance of the tissue is less than that of the model at a given time, more current will flow through the arm of the bridge containing the tissue than through the other, and vice versa.

We are unable to account for the discrepancy between our observations and those of Rapport and Ray (1927). The electrodes in their study were placed one in Ringer solution filling the manometer connected to the cannulated ventricle and the other in a solution bathing the external surface. We tried this method but discarded it because we could only record very minor changes of impedance. This difficulty was expected, for the arrangement is such that the ventricle is effectively in series with a relatively high resistance—that of the Ringer solution intervening between the tissue and the electrodes. We found further that twisting of the ventricle during contraction, or without a contraction, could result in marked changes of conductivity, due probably to changes in the degree of occlusion of the orifice of the cannula.

Since more than 100 observations in 20 hearts were consistent in showing an increase of impedance during activity, and since a decrease of impedance was only registered when movement was obvious at or between the electrodes, it is concluded that an increase is genuine and a decrease is an artifact.

Dubuisson (1937) has described two separate components in the impedance changes during contraction of striated muscle. These 2 components would result in 2 peaks in the impedogram, one during the phase of rising tension, the other during relaxation. Impedograms with 2 peaks were seen in these observations only when there was obvious movement, and hence an obvious source of artifact (p. 517). A complex impedogram might be expected because of the complexity of the ventricular response. Thus, it would seem likely that both the excitatory and conducting processes on the one hand, and the contractile mechanism on the other, would be associated with independent variations of the conductance of the tissue. The present data do not confirm this expectation.

In nerve, where there is no contractile mechanism but only excitation and conduction, Cole and Curtis (1939) have shown that there is a decrease of impedance associated with the passage of a nerve impulse. This decrease starts shortly after the beginning of the spike potential and reaches a maximum approximately at the same time as the spike. It was expected, therefore, that there would be a similar initial drop of impedance in the ventricle. The record in figure 3A does not confirm this expectation. If there is an initial drop it is very small, compared to the later rise of impedance.

The significance of the variations of impedance during activity can not be determined with the data available. Since these variations outlast the changes of tension, a recovery process is suggested (see Dubuisson, 1937). Whether this recovery is associated with conduction, or with contraction, or with both, remains to be elucidated. When the mechanical events are briefer the impedance changes are also briefer (fig. 1A and D), but, as is well known, the electrical responses are also shorter in these conditions.

SUMMARY

The changes of electrical impedance during the activity of the turtle's ventricle were measured by means of an alternating current bridge and recorded from a cathode-ray oscillograph.

The impedance to alternating currents of 30 to 10,000 cycles per sec. and of 0.04 to 0.06 ma. increases during activity. The increase begins approximately at the same time as contraction, but it outlasts the mechanical events (fig. 1A and B). There is no parallelism between the impedogram and the electrogram (fig. 1A and C). Brief electrical and mechanical responses involve a brief impedance variation (fig. 1D).

Upon repetitive stimulation a long enduring impedance change may ensue (fig. 2).

The early part of the ventricular electrogram is not attended by any striking change of impedance (fig. 3).

REFERENCES

BISHOP, G. H. This Journal **89**: 618, 1929.
BOZLER, E. J. Cell. and Comp. Physiol. **6**: 217, 1935.
BOZLER, E. AND K. S. COLE. Ibid. **6**: 229, 1935.
COLE, K. S. Cold Spring Harbor Symposia VIII, 1940.
COLE, K. S. AND H. J. CURTIS. J. Gen. Physiol. **22**: 649, 1939.
DUBUISSON, M. J. Physiol. **89**: 132, 1937.
RAPPORT, D. AND G. B. RAY. This Journal **80**: 126, 1927.

A COMPARISON OF ATROPINE AND CURARE AS ANTAGONISTS OF ACETYLCHOLINE[1]

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The action of acetylcholine (Ach.) is usually considered to be nicotinic or muscarinic, depending on whether its effect is inhibited by atropine or curare, respectively.

Not all experimental data fit this formulation. Clark and Raventós (1937) observed *in vitro* that curarine and atropine blocked the effect of Ach. both for the auricle and the rectus abdominis of the frog. Marrazzi (1939) showed that in the rabbit the nicotinic effects (superior cervical ganglion) are antagonized by atropine. On the other hand, Luco and Meza (1941) established that curare blocked the connection between the parasympathetic postganglionic nerve and the smooth muscle.

Abdon (1940) stated that there was a constant ratio between the amount of Ach. necessary to produce a definite effect in the frog (heart inhibition, contraction of the rectus abdominis) and the amount of atropine capable of abolishing the effect. The object of the present work is to study in mammals the relationship between Ach. and curare. An attempt will also be made to interpret the differences found between the muscarinic and nicotinic effects of Ach. in the consideration of both groups of results.

METHODS. Cats were anesthetized with diallylbarbituric acid (Dial Ciba), 0.75 cc. per kgm. The response of the submaxillary gland was determined by the number of drops delivered from a glass cannula placed in Wharton's duct. The contraction of the superior rectus muscle of the eye was recorded on a kymograph with an amplification varying between 5 and 7, after removal of the eyeball. The muscle and the gland were decentralized by section of the common oculomotor nerve in its intracranial region and of the chorda tympani, and by extirpation of the ciliary and superior cervical ganglia. In some experiments the chorda tympani was stimulated with a Harvard inductorium. The injections were made into the common carotid artery. The drugs were from the following sources: acetylcholine hydrochloride (Hoffmann La Roche); atropine sulphate (Mallinckrodt); and curare (Merck), an empirical solution which was standardized biologically.

RESULTS. In all experiments the responses of muscle and gland were recorded simultaneously. The smallest amount of Ach. capable of inducing the minimum response of each effector was determined, using as end-points one or two drops from the gland and a definite movement of any part of the muscle. In each experiment the regularity of effect of the same stimulus was determined carefully, with injections of Ach. given at regular time intervals.
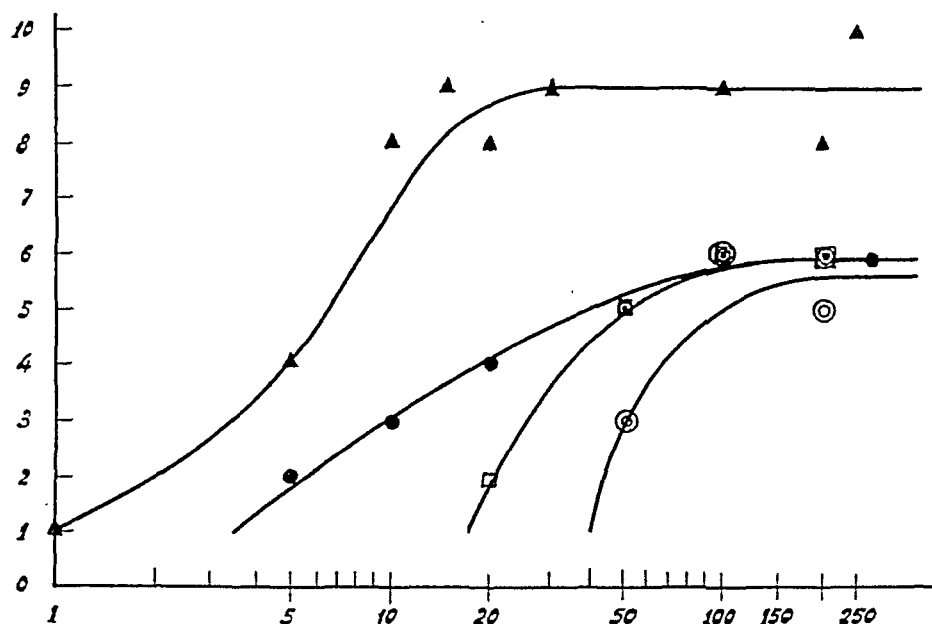
Fig. 1. Effect of curare on the response of the submaxillary gland to acetylcholine: Abscissae: dose of Ach. in micrograms (log. scale). Ordinates: intensity of response measured by the number of drops (arithmetic scale). Double circles, squares, filled-in circles: 1 minute, 17 minutes and 33 minutes, respectively, after injection of curare.

TABLE 1

The dosages of Ach. are the threshold values for each experiment; those of atropine and curare are the least amounts capable of blocking the responses to the respective threshold stimuli. Dosages are in micrograms. The letters M and G stand for muscle and gland. The designation — means that the corresponding dose was not determined. Dosage of curare is in cubic centimeters.

NO.	ACETYLCHOLINE		ATROPINE		CURARE	
	M	G	M	G	M	G
1	5	1	4,000	—		
2	10	1	11,000	—		
3	10	0.5	1,000	0.1		
4	10	1	6,000	1.1		
5	10	1	5,000	0.2		
6	5	1	4,000	1.0		
7	5	1	11,000	0.1		
8	5	1	6,000	1.0		
9	7.5	1			0.1	0.6
10	5	1			0.1	1.1
11	10	1			0.1	0.6
12	10	1			0.1	0.9
Average..........	7.708	0.958	6,000	0.583	0.1	0.80
Standard Deviation............	±0.707	±0.041	±1,224	±0.170	±0.0	±0.122

1. *Atropine and skeletal muscle.* The effect of atropine on the response of the muscles of the eye to acetylcholine has been studied previously. Duke-Elder and Duke-Elder (1940) injected atropine intravenously (5,000 micrograms) and found that this drug did not affect the response in question.
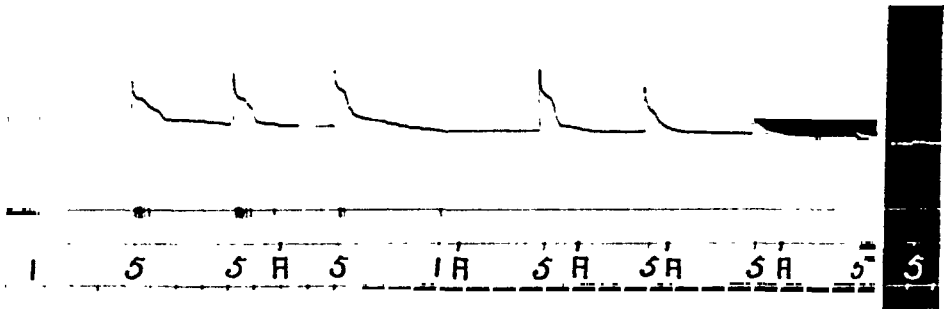
Fig. 2. Antagonism between acetylcholine and atropine. Upper graph: muscle. Upper line: secretion of the gland in drops. Middle line: the numbers indicate the doses of Ach. (micrograms). *A* represents the injections of atropine: 0.1, 0.9, 1,000, 1,000 and 1,000 micrograms of atropine successively. Twelve minutes elapse between the two sections of the figure, during which 3,000 micrograms of atropine were injected. Time in minutes.
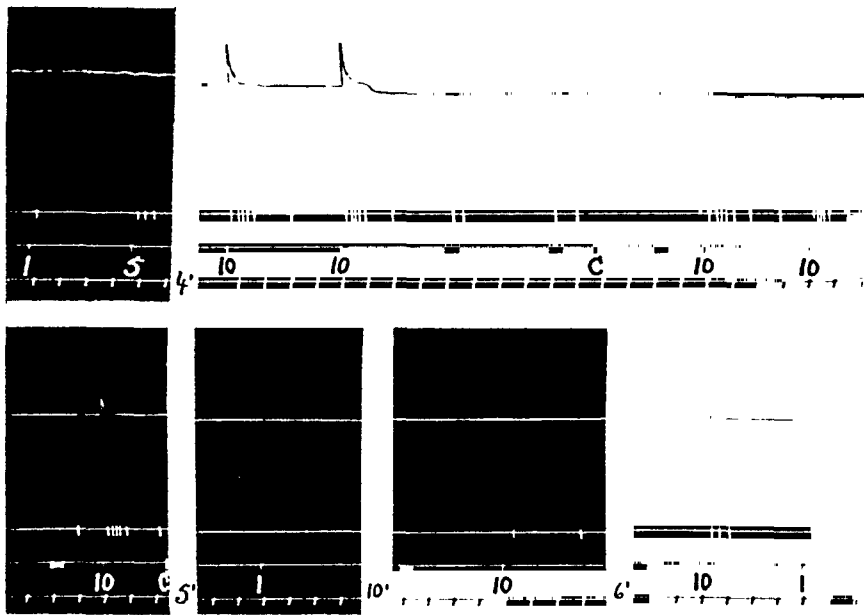
Fig. 3. Antagonism between acetylcholine and curare. Upper graph: muscle tracings. Upper line: glandular response. Intermediate line: the numbers stand for dosage of Ach. in micrograms; at the first *C*, 0.1 cc. of curare was injected; at the second *C*, 0.6 cc.; the other signal indicates the electrical stimulation of the chorda tympani. Lower line: time in minutes. The numbers between the lines indicate the elapsed time.

Our results indicate that it is inhibited by high dosages of atropine (fig. 2 and table 1).

2. *Curare and glandular responses.* Luco and Meza (1941) demonstrated that curare in large doses blocks transmission between the electrically-stimulated

parasympathetic postganglionic nerve and the corresponding effector; they suggested that curare inhibits all transmission of the cholinergic type, each system requring a different concentration.   This hypothesis led to the supposition that the submaxillary gland, stimulated by Ach., could be curarized.   Accordingly, the response of the gland as a function of the concentration of Ach. (intensity of stimulus) was studied in a group of animals, and also the modifications produced by curare.   Figure 1 is a typical example of these experiments.

The normal curve of action-concentration is of the usual type.   Curare raised the threshold to Ach. and limited the maximum response of the gland.   Spontaneous decurarization is characterized by the enduring limitation of response, while the threshold returns to the normal value.

3. *Atropine and Ach.*   During the period of stimulation with Ach., atropine was injected in varying dosage (between 0.1 and 11,000 micrograms, always in a volume of 0.2 cc.), noting the minimal paralyzing doses for the nicotinic and muscarinic responses, respectively.   The results of each experiment are summarized in table 1.

If the amounts of atropine capable of inhibiting the responses of muscle and gland are compared, it will be seen that the doses are several thousand times greater in the first case than in the second (fig. 2).

4. *Curare and Ach.*   In the same way as in the previous series, a comparative study was made of the minimum dose of curare necessary to block the effectors described above.

In this case, the results obtained were opposite to those of series 2, i.e., muscle (nicotinic effect) is inhibited by a dose of curare much smaller than that needed to inhibit the gland (muscarinic effect, fig. 3).

DISCUSSION.   A. *Technique.*   The superior rectus muscle of the eye was used as an effector of the nicotinic type, and the submaxillary gland was selected as an effector of the muscarinic type, since they offer the following advantages over other effectors: 1. The extrinsic muscles of the eye are very sensitive to Ach. and thus the need for the injection of large doses is avoided, which may cause secondary effects.   2. The simultaneous stimulation of both effectors is very easy to carry out by means of intracarotid injections.   3. The short distance from the site of injection assures the constancy of the intensity of stimulus in both effectors.

Extirpation of the parasympathetic ganglionic system is very difficult, and therefore it was not done in any experiment.   This renders difficult the interpretation of some results; Ach. not only stimulates the gland directly, but also in part through the ganglion.   A similar argument applies to the inhibitions by both curare and atropine.

In order to evaluate the importance of the ganglion, a series of experiments were carried out in which the response of the gland was recorded following electrical stimulation of preganglionic parasympathetic fibers.   Figure 3 shows a typical result in this series.

Stimulation of the chorda tympani induces a constant response of the gland. A small dose of curare blocks the response to an equal stimulus, without altering

the effects of a given dose of Ach. This demonstrates the slight importance of the ganglion in glandular stimulation by Ach.

B. *Results.* The observations cited in the introduction and the results of this work suggest the doubtful validity of the division of acetylcholine functions as muscarinic and nicotinic.

On inspection of the experiments with curare and atropine, it will be noted that the results are inverse, i.e., an effector with a high threshold for Ach. is blocked by large doses of atropine or small doses of curare; conversely, an effector with a low threshold is inhibited by high levels of curare or low doses of atropine.

If one accepts the general hypothesis that one or more processes occur between the stimulus and the response, these being the fixation of the drug and its selective action after fixation, as believed by Clark (1937), or that some other phenomenon may occur, the results described cannot be interpreted on a basis of there being only a quantitative difference between the muscarinic and the nicotinic effects. It is necessary to postulate at least the existence of two quantitatively different, but qualitatively equal, processes, and of necessity atropine acts on one and curare on the other.

The only other possible interpretation is that one or more processes are qualitatively different.

SUMMARY

The relationship between the acetylcholine-curare and acetylcholine-atropine antagonisms was studied in cats anesthetized with Dial as a function of the varying thresholds to acetylcholine exhibited by the superior rectus muscle of the eye and the submaxillary gland.

Large doses of atropine inhibit the muscular response to acetylcholine (fig. 2 and table 1).

Large doses of curare block the glandular response to acetylcholine (figs. 1 and 3, table 1).

The doses of atropine capable of inhibiting the responses to acetylcholine are directly proportional to the acetylcholine thresholds of the effectors used. With curare this relationship is reversed.

In the discussion, an explanation is offered for the differences between the nicotinic and muscarinic activities of acetylcholine.

REFERENCES

ABDON, N. O. Acta Physiol. Scand. 1: 153, 1940; cited by BULBRING AND BURN. J. Physiol. 100: 337, 1941.

CLARK, A. J. General pharmacology. Heffter's Handbuch exper. Pharmacol. Ergänzungswerk 4. Julius Springer, Berlin, 1937.

CLARK, A. J. AND J. RAVENTÓS. Quart. J. exper. Physiol. 26: 375, 1937.

DUKE-ELDER, W. S. AND P. M. DUKE-ELDER. Proc. Roy. Soc. London B 107: 332, 1930.

LUCO, J. V. AND J. MEZA. Ciencia (México) 2: 298, 1941.

MARRAZZI, A. S. J. Pharmacol. and Exper. Therap. 65: 18, 1938.

RESULTS OF PREFRONTAL LOBECTOMY ON ACQUIRED AND ON ACQUIRING CORRECT CONDITIONED DIFFERENTIAL RESPONSES WITH AUDITORY, GENERAL CUTANEOUS AND OPTIC STIMULI[1]

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This, the third study on the functions of the association cells of the cerebral cortex, is concerned with the effect of prefrontal lobectomy on acquired and acquiring correct conditioned differential responses of the foreleg from auditory, general cutaneous and optic stimuli. It will be seen that the results though negative in nature attain special interest when compared with earlier work on olfactory stimuli (1940) and with some preliminary unpublished work on these analysers after removal of large areas of the posterior cerebral association cells.

The term *correct conditioned differentiation* has been explicitly defined in earlier papers, in which the emphasis was placed on the correctness of the positive and negative conditioned responses rather than on fine discriminations. The same general procedures and controls were also used. The dogs represented the easily excited, easily inhibited and the evenly balanced type of cerebral cortex. Some of them 1, 3 and 4 were used in the previous report (1943), with which identical tests can be compared after prefrontal lobectomy. Ordinarily a two week recovery period preceded the first tests.

The results of these differential tests are summarized in tables and each table shows the records of two different conditioned differential tests for a common stimulant, one based on specific properties and the other on varied intervals of the same stimulant. In the first left column the dogs are listed by number, the first three being tested before and after prefrontal lobectomy and the others only after the extirpations. The second and third columns include the results of a single day's tests[2] after differentiation was standardized; the ratios of correct (C) to incorrect (I) for the positive conditioned tests appear in column 2, while the ratios of the incorrect (I) to correct (C) for the negative conditioned tests appear in column 3. Columns 4 and 5 are similar to 2 and 3 but include a record of all positive and negative conditioned reflex tests arranged for correctness and errors. A predominance of incorrect responses in these columns indicates difficulties encountered in acquiring correct differential responses. In these cases the negative tests ordinarily resulted in positive responses more often than the

positive tests revealed an absence of response. The fifth or last column shows the grade of correct differential responses obtained.

Auditory stimulus. Three sets of differential tests were routinely used. In (1) a bell and a board tapped once per second served for the positive and negative stimuli. In (2) an iron cup was substituted for the board. In (3) the bell was tapped once per second for the positive reflex and three times per second for the negative reflex.

TABLE 1

Results of auditory stimuli after prefrontal lobectomy

DOG	BELL 1 PER SEC. POS. REF. A day's tests after diff. standardized		BOARD 1 PER SEC. NEG. REF. Total number of tests		DIFF.	BELL 1 PER SEC. POS. REF. A day's tests after diff. standardized		BELL 3 PER SEC. NEG. REF. Total Number of tests		DIFF.
	Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C		Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C	
Series 1. Tested before and after ablations										
1	24–1	0–12	65–1	7–81	good	12–0	3–22	60–1	9–41	good
3	32–0	1–9	176–12	46–60	good	24–1	2–11	100–15	19–23	good
4	12–0	0–25	60–2	2–46	good	13–0	1–24	140–5	30–81	good
						25–0	0–12			
Series 2. Tested after ablations										
2	24–1	2–8	148–4	43–24	fair	27–0	1–8	181–5	66–25	good
5	29–0	2–24	106–2	11–75	good	24–1	1–7	168–1	79–19	good
6	25–0	1–11	75–0	6–22	good	25–0	15–0	164–3	203–1	none
7	24–1	0–8	66–12	1–24	good	11–1	0–25	163–39	33–138	good
8	12–0	1–25	188–12	29–195	good	25–0	0–12	130–0	16–57	good
	25–0	0–12								

Abbreviations used in table

Diff. = Correct conditioned differential responses.
Pos. ref. = Positive conditioned reflex; Neg. ref. = Negative conditioned reflex.
Pos. = Positive conditioned tests; Neg. = Negative conditioned tests.
C to I = Ratio correct to incorrect; I to C = Ratio incorrect to correct.

It is clear from columns 2 and 3 of the left half of table 1 that dogs 1, 3 and 4, which were able to respond correctly with the conditioned differential tests with the bell and board before prefrontal lobectomy (table 1, previous paper), were still able to make a perfect or practically perfect score of a day's test after prefrontal lobectomy. The total number of tests (columns 4 and 5) also discloses a good percentage of correctness for dogs 1 and 4 after prefrontal lobectomy During the first session after operation dog 1 made 4 errors in 25 negative tests and none in 12 positive tests, while dog 4 made 2 errors in 25 positive tests and none in 12 negative tests. The first positive and negative tests were correct for both dogs. Dog 3 did very well for the second day's ordeal, then poorly for 2 sessions, to become practically perfect afterward.

The second group of dogs 2, 5, 6, 7 and 8 not subjected to any differential tests until after prefrontal lobectomy, reveals (columns 2 and 3 of the left half of table 1) smilar daily records for the bell and board to the group which was trained before the operation. The fact that the total number of tests (columns 4 and 5) discloses a general tendency toward correct responses for dogs 5, 6, 7 and 8, while dog 2 shows none, is to be attributed to a longer learning period for this dog. The number of daily sessions required of dogs 2, 5, 6, 7 and 8 before the first signs of differential responses appeared were 4, 2, 3, 1 and 1 days respectively. Since it required 2, 1 and 1 day's testing for the first group to

TABLE 2

Results of general cutaneous stimuli after prefrontal lobectomy

DOG	WITH HAIR GRAIN 1 PER SEC. POS. REF. A day's tests after diff. standardized		AGAINST HAIR GRAIN 1 PER SEC. NEG. REF. Total number of tests		DIFF.	WITH HAIR GRAIN 1 PER SEC. POS. REF. A day's tests after diff. standardized		WITH HAIR GRAIN 3 PER SEC. NEG. REF. Total number of tests		DIFF.
	Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C		Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C	
Series 1. Tested before and after ablations										
1	24–1	0–12	110–2	0–73	good	25–0	1–11	160–0	18–121	good
3	25–0	0–12	149–2	22–85	good	25–0	0–12	124–1	21–79	good
4	13–0	0–25	63–0	4–45	good	25–0	0–12	39–0	1–36	good
Series 2. Tested after ablations										
2	24–1	3–9	106–1	25–35	fair	22–3	0–12	46–4	1–16	good
5	1–11	25–0	34–57	219–29	inver.	25–0	0–9	46–4	1–14	good
7	24–1	1–10	176–22	28–108	good	11–1	0–25	139–9	21–102	good
8	25–0	0–12	108–4	5–68	good	25–0	0–12	37–0	0–37	good
	12–0	0–25								

Abbreviations used in table

Diff. = Correct conditioned differential responses.
Pos. ref. = Positive conditioned reflex; Neg. ref. = Negative conditioned reflex.
Pos. = Positive conditioned tests; Neg. = Negative conditioned tests.
C to I = Ratio correct to incorrect; I to C = Ratio incorrect to correct.

demonstrate the first signs of correct differential responses before prefrontal lobectomy, this group of dogs required but slightly more training than normal dogs.

After prefrontal lobectomy both groups of dogs made as high scores for a day's session with the bell and cup as they did with the bell and board and this was accomplished with the first or second day's tests. This excellent record with a similar but more difficult discrimination was obviously due to having followed the bell and board tests.

The results of both groups of dogs with the third set of sound analysers, namely, bell tapped once per second and three times per second are recorded

in the right half of table 1. Columns 2 and 3 show that with the exception of dog 6 both groups of dogs ultimately made as perfect daily records with this set of analysers as with the bell and board. After making due allowance for previous training, group 1 made a much better total record for correct responses than group 2 (columns 4 and 5). Dogs 1, 3 and 4 acquired the first signs of correct responses for the slow and fast bells in 2, 1 and 2 days of testing before prefrontal lobectomy and afterward in 2, 3 and 1 days respectively; while dogs 2, 5, 6, 7 and 8, not tested until after operation, required 6, 7, ?, 4 and 1 day of testing. Dog 8 while showing signs of correct differential responses during the first day's ordeal revealed no further signs of correct responses until the fourth day's tests. Dog 6 in not being able to respond correctly in 11 days of testing was unable to hold back his positive foreleg responses during 203 negative tests followed by punishment and intermixed with 167 positive tests, three of which were incorrect and punished.

Not only were all the prefrontal lobectomized dogs able to respond correctly to all three sound tests in varied ratios, but they were able to respond correctly to positive and negative conditioned tests alternated 4 to 6 times with intervals of 3 seconds between tests.

A more difficult sound conditioned differential test was attempted toward the end of the problem, unfortunately with but one dog and only after prefrontal lobectomy. The test was somewhat similar to one used successfully by Babkin with unoperated dogs. The positive stimulus consisted of tapping a bell once, then a second's pause, followed by tapping the bell 3 times per second and the negative stimulus consisted of tapping the bell 3 times per second, a second's pause, followed by 1 tap of the bell. The dog used was no. 8, the most likely of the prefrontal lobectomized dogs to acquire correct responses with this differential test. Out of 225 positive tests 215 were correct and of 298 negative tests 176 were positive and incorrect in spite of a whipping for each error. Respiratory tracings taken during negative tests that resulted in positive responses afforded other examples of 2 irradiated cortical waves, one excitable and incorrect and two, inhibitory and correct.

General cutaneous stimuli. Two sets of differential tests were used for this sense. In the first, the hair of the back was stroked with a hand brush once per second with the grain and once per second against the grain for the positive and negative conditioned reflexes. In the second set, the hair of the back was stroked with the grain once per second and three times per second for the positive and negative reflexes. The same groups of dogs were used in these tests as were used for sound. Group 1 (dogs 1, 3 and 4) was trained to make correct differential responses before prefrontal lobectomy; while group 2 (dogs 2, 5, 6, 7 and 8) was not tested until after the operation. Table 2 follows the same general plan for recording the results as was used for sound in table 1.

Columns 2 and 3 of both halves of table 2 show that the first group of dogs was able after prefrontal lobectomy to make perfect or practically perfect scores with both sets of general cutaneous stimuli for a day's test. Both before and after prefrontal lobectomy these dogs required only 1 or 2 days' testing to elicit

correct foreleg responses for either set of differential tests. After prefrontal lobectomy only dog 1 required a second session to acquire correct responses and it was for the tests based on rate of the stroke. This accounts for the good showing of the total tests in columns 4 and 5 of both sides of table 2.

Likewise columns 2 and 3 and 4 and 5 of both sides of table 2 show that the second group of dogs 2, 5, 7 and 8 (not trained before prefrontal lobectomy) have obtained perfect daily records for both general cutaneous tests after the operation and that a large number of the total tests were correct. The earliest signs of correct differential responses for the first set of general cutaneous tests (with and against the grain) appeared during the first day's tests with dogs 7 and 8, during the second with dog 2, but not until the fourth day for dog 5 and then the positive and negative reflexes were inverted.[3] The first signs of correct responses for the second set of differential tests appeared during the first day's testing with all dogs of the second group. It should be mentioned while dog 8 demonstrated signs of correct differential responses during the first session it was not until the fourth session that correct responses reappeared.

Both groups of dogs with the exception of dog 5 (not tried) were able to respond correctly to both sets of general cutaneous tests when the positive and negative conditioned tests were alternated 4 to 6 times with intervals of 3 seconds between tests.

A third set of general cutaneous tests was made on dogs 1, 3 and 4 in which inhalation of chloroform and acetic acid vapors served for the positive and negative conditioned reflexes. In these experiments the olfactory tracts were severed to eliminate olfactory stimulation. These dogs which were able to respond about 75 per cent correct before prefrontal lobectomy responded equally well afterward.

Optic stimuli. Dogs 1, 3 and 4, previously trained to average about 75 per cent correct differential responses before prefrontal lobectomy (1943, table 4) with two different sets of optic analysers, namely, light contrasted with black screens and constant light against a flicker (1 per second) were able to maintain about the same average of correctness after prefrontal lobectomy.

Lesion and autopsies. The lesion started directly in front of the precruciatus sulcus (supraorbital of Langley) and if the olfactory tract was to be severed extended to the base of the skull. It involved no difficulties, since the only precaution was to avoid slanting the spatula caudad to include the motor projection fibers.

Dogs 4 and 8 have not been autopsied for the reason that they are being used for another problem. The brains of the others have not only been carefully

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[3] After prefrontal lobectomy dog 5 acquired the positive conditioned reflex of stroking the hair of the back with the grain during the first day's tests. Throughout the first three days' sessions for differential reflexes with and against the grain, all of the positive and negative tests resulted in positive responses. During the fourth session practically all the positive tests resulted in absence of response and nearly all the negative tests continued positive. These inverted reflexes continued throughout the remaining tests in spite of a total of 57 shocks and 219 whippings.

studied, but transections have been made through the medulla stained after the Marchi method to show pyramidal degeneration.

Complete ablation of the prefrontal areas was obtained in every instance with the exception of 5 to 7 mm. of the caudal end of the orbital lobe, namely, the region immediately behind the interorbital fissure (Langley's fig. 1, i.or.). There was slight injury to the median cephalic surface of the left sigmoid gyrus of dog 2, but Marchi sections through the medulla of this dog disclosed only a few more than the normal number of degenerated fibers in the corresponding pyramid. The brains of the other dogs showed no injuries to the motor cortex, no more than the normal amount of degeneration in the pyramids and none of the dogs exhibited motor symptoms.

DISCUSSION. Since prefrontal lobectomy did not prevent the rapid appearance and perfect performance of certain conditioned differential responses from several different auditory and general cutaneous stimuli in dogs that were trained before and after the operation, its effects are obviously very different from the effect previously reported for olfactory stimuli. These results though negative in nature are of equal importance to the positive results of this ablation on correct differential olfactory conditioned responses for they suggest a lesser independence on the prefrontal or anterior association and correlation cells for correct analysis, synthesis, associated memory and the correct use of inhibition and excitation. It may be noted in this connection that a few preliminary experiments indicate a greater use of the posterior association cells surrounding the auditory, general cutaneous and optic projection centers (a large part of the sensory cortex of Dusser de Barenne, McCulloch, Garol et al.) for correct auditory and general cutaneous conditioned differential responses.

It might be supposed since the positive conditioned stimulus was the same for all of the auditory, and for all of the general cutaneous tests that the last set of differential analysers used would be those in which correct responses would be obtained with the least number of trials. This happened to be true for the general cutaneous analysers but not for the auditory. It is not strange that dog 3 of the first group and dogs 2, 5 and 7 of the second group had more difficulty with some differential tests than with others. Why all of the dogs of the first group were not able to make correct differential responses with the first tests is difficult to explain. It cannot be due to faulty circulation or edema of the posterior association and correlation cells or the posterior inhibitory areas because the positive conditioned reflexes appeared with the first trials and they are contingent on the functioning of the motor cortex which is situated directly behind the lesion. While the early errors are chiefly shown in the negative conditioned tests, cortical inhibition is not absent, for its presence is demonstrated in respiratory tracings taken at the time of incorrect positive tests and some correct negative tests. What is lacking is *correct* inhibition. The immediate delay of correct responses may be due to something akin to shock of the entire association cell system.

It is apparent that these results are at variance with Babkin's (quoted by Pavlov and by Eliasson) in which it was found that prefrontal lobectomy clim-

inated the positive conditioned cutaneous reflex from all parts of the body except the limbs.

It is singular in view of the results of others that Ten Cate was not only able to obtain conditioned sound reflexes but some differentiation between sounds after complete removal of the neopallium in the cat. It is however possible that the afferent and efferent connections between the cerebrum and the thalamus as indicated by Dusser de Barenne et al. and by Morison and Dempsey also contribute to the higher cortical activities.

SUMMARY

With one exception extirpation of both prefrontal areas produced no permanent effect on acquiring or on acquired correct conditioned differential responses elicited from somewhat similar tests with auditory, general cutaneous and optic stimuli.

Correct conditioned differential reflexes were also obtained from both groups of dogs after prefrontal lobectomy from sound and general cutaneous stimuli when the positive and negative tests were alternated 4 to 6 times with intervals of 3 seconds between tests.

After making due allowance for individual differences for certain tests and that the average dog had more difficulty in responding correctly to differential tests based on rate of a given analyser than on its various components, it is apparent that 2 of the 5 dogs, which were not tested until after prefrontal lobectomy, required more tests than normal dogs to respond correctly with the 1 and 3 second sound tests and one dog was unable to acquire the same in the time allotted for the tests.

The most promising of the second group of prefrontal lobectomized dogs did not acquire correct responses to a more complicated sound differential test.

REFERENCES

ALLEN, W. F. This Journal **121**: 657, 1938.
   Ibid. **128**: 754, 1940.
   Ibid. **132**: 81, 1941.
   Ibid. **136**: 783, 1942.
   J. Gen. Psychol. In press.
BABKIN, B. P. Bull. Milit. Med. Acad. 1909, Quotation.
   Proc. Rus. Med. Soc. Petrograd **77**: 1910, Quotation.
CULLER AND METTLER. J. Comp. Psychol. **8**: 291, 1934.
DEMPSEY AND MORISON. This Journal **138**: 283, 297, 1943.
DUSSER DE BARENNE ET AL. J. Neurophysiol. **1**: 69, 1938.
   Ibid. **4**: 287, 304, 311, 324, 1941.
ELIASSON, M. Arch. f. Ohren-Nasen u. Kehlkopfheilkunde **139**: 263, 1935.
FROLOV, Y. P. Pavlov and his school, 1937.
GAROL, H. W. J. Neuropathol. and Exper. Neurol. **1**: 139, 320, 422, 1942.
LANGLEY, J. N. J. Physiol. **4**: 286, 1884.
PAVLOV, I. P. Conditioned reflexes. Transl. G. V. Anrep, 1927.
   Lectures on conditioned reflexes. Transl. W. H. Gantt, 1938.
   Conditioned reflexes and psychiatry. Transl. W. H. Gantt, 1941.
TEN CATE, J. Arch. Néerland. de Physiol. **19**: 191, 1934.

FACTORS INFLUENCING MICTURITION VOLUME IN THE RAT

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It is well known that after the fourth month of pregnancy, in human beings, the bladder increases in size and rises into the abdomen. During this period a condition of smooth muscle atonia develops which is made evident not only by relaxation of the bladder wall but also by relaxation of the ureters, uterus, large bowel and bile duct. Following parturition the bladder continues in a distended hypotonic state for a period of about six weeks, gradually returning to normal (1, 2, 3). Various theories have been advanced to explain these phenomena but little has been done to test the validity of the hypotheses.

Woolsey and Brooks (4) noted that marked increases in micturition volume occurred in cats post-partum. They also observed changes in micturition volume in cats during spontaneous estrus, during pregnancy and following injection of alpha-estradiol benzoate. Langworthy and Brock (5), using a different technique, found bladder hypotonicity to develop in the pregnant and post-partum rabbit and suggested that progestin might be responsible for the observed loss of bladder tone. We have attempted to obtain further evidence concerning the rôle played by hormonal factors in producing the changes in bladder responses known to occur during pregnancy and following parturition. Rats were used in this study because of their ability to rear litters at frequent intervals under ordinary laboratory conditions.

METHODS. Two techniques were employed in measuring bladder response and micturition volumes. In many instances both were used on the same animal to measure the changes in bladder reactions occurring during pregnancy and following parturition or hormone injections.

First, by means of kymographic tracings taken from the apparatus pictured in figure 1-A we were able to determine not only the total daily urine volumes but also the volume of urine eliminated during each micturition. The sensitivity of this device was such that 0.1 cc. of urine produced a 5 mm. excursion of the writing point. The daily water and food intake was measured; estrous cycles were followed and the animals were weighed once a week. Twenty-four females and four males were studied during many weeks and the effects of various hormones on bladder responses determined. Eighteen females were maintained in the apparatus throughout pregnancy and the subsequent period of lactation and nursing.

The second type of procedure permitted measurement of the bladder volume and intravesicular pressure at which micturition occurred in anesthetized ani-

mals. We used an apparatus similar to that devised by Langworthy, Reeves and Tauber (6) and Schultz (7). After establishment of light ether anesthesia the rat's bladder was exposed and an 18 gauge hypodermic needle, connected by pressure tubing to the manometers and fluid reservoir, was pushed through the bladder wall and used to introduce warm saline into the viscus. Although the head of pressure was constant (90 cm. of water) the rate of bladder filling varied due to differences in bladder resistance to distention. Filling was continued until fluid escaped through the urethra. The volume and intravesicular pressure at which escape occurred were considered to be the characteristic micturition volume and pressure of that particular bladder. During each test period results
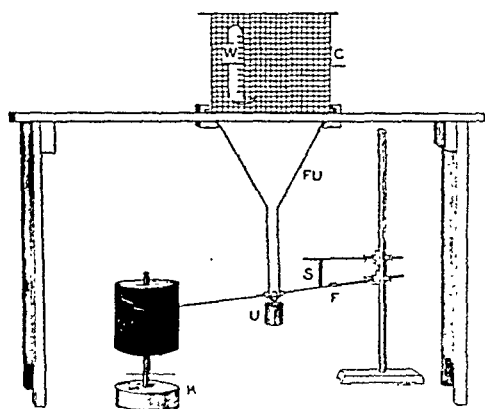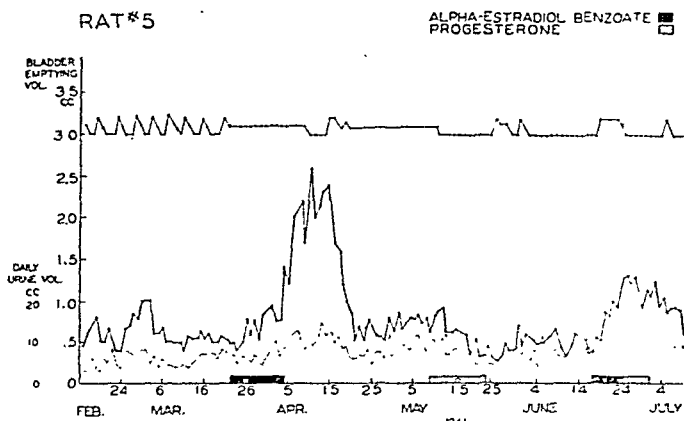
Fig. 1-A   Fig. 1-B

Fig. 1-A. Apparatus for recording micturition volume of an unanesthetized rat. Cage, c, and water cup, w, placed on a stand above a funnel, fu, delivering urine to a cup, u, which is attached to a writing point recording on a slowly moving kymograph, k. The position of the fulcrum, f, and supporting spring, s, is such that very small increments in cup weight are recorded.

Fig. 1-B. Chart showing how average daily micturition volumes, average daily urine volume and estrous cycles of rat 5 were affected by injections of estrogenic material and progesterone. First injection—200 rat units daily for 12 days; 2nd injection—1 mgm. progesterone daily for 14 days; 3rd injection—200 rat units of estrogenic material for 7 days followed by 1 mgm. progesterone daily for 7 days.

obtained were checked by refilling the bladder to the micturition threshold four to six times. The hypodermic needle was then withdrawn and the incision closed. The same animal could thus be tested before pregnancy and before hormones were injected, during and after recovery from the effects of hormone injections and other procedures.

RESULTS. 1. *Normal animals.* Results obtained on unanesthetized animals with the apparatus shown in figure 1-A revealed that, as in other species (2), each rat had a characteristic normal micturition volume. Female rats had, on the average, greater bladder volumes than did the males. Large rats tended to have larger bladder capacity than did smaller individuals but this was not invariably true. A comparison of the weight of the filled bladder to the body weight showed that the bladder comprised 0.15 per cent of the body weight in some individuals and 0.87 per cent in others. In the vast majority of cases the bladder normally

emptied when filled to such a volume that it constituted 0.25 to 0.30 per cent of the total body weight. In one group of six rats the individual body weights approximated 240 grams but their characteristic micturition volumes ranged from 0.42 cc. to 0.89 cc. The average micturition volumes of unanesthetized non-pregnant mature rats normally varied between 0.45 cc. and 0.65 cc. Some idea of the constancy maintained within the individual can be obtained from figure 2.

Anesthetics, or the procedures attendant upon the method of artificial filling, rendered the bladder much less responsive. When the same animals were used
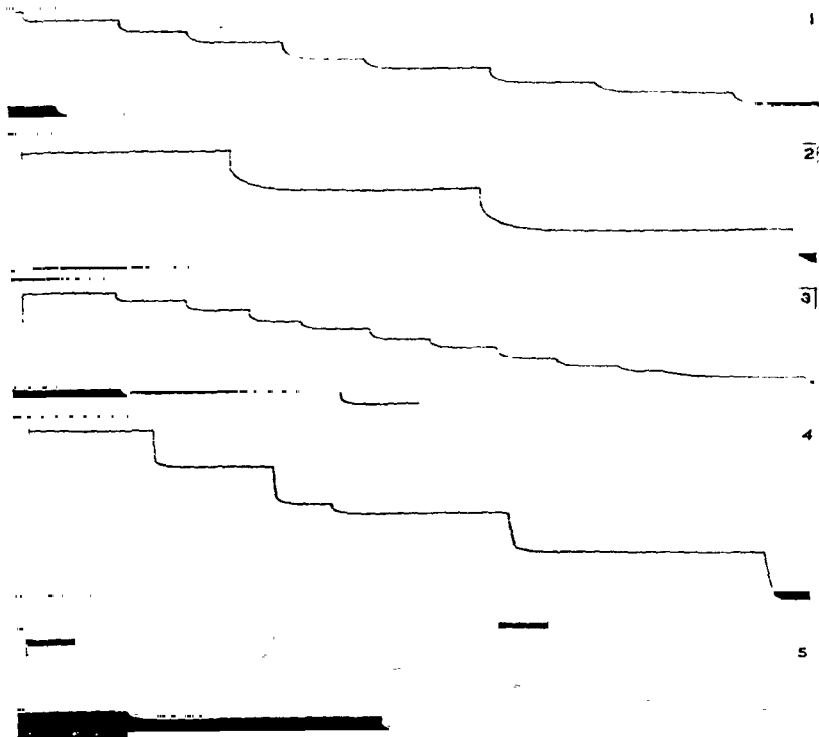
Fig. 2. Actual records taken with apparatus shown in figure 1-A. Records of rat 3 whose total history is shown in figure 3. (1) Normal—March 25. (2) Nursing—April 18. (3) Normal—May 16. (4) After estrogen injection—June 15. (5) Normal—July 20 (not shown in fig. 3).

which gave the above-stated averages in the unanesthetized state, micturition did not occur until a volume of approximately 0.9 cc. was reached. With this technique individual differences in the bladder tone of normal rats were revealed by the fact that despite a uniform head of perfusion pressure the rates of filling varied between 0.16 and 0.67 cc. per minute (average 0.43 cc.). The average intravesicular pressure at which micturition occurred was 30 cm. of water. Our results were similar to those obtained by Schultz (7).

2. *Pregnancy.* A study was made of bladder responses throughout sixteen pregnancies in a total of eleven rats. During pregnancy there was generally a gradual increase in micturition volume (fig. 3). The time of appearance of this rise varied enormously as did the magnitude of the changes. The rise began on

the second day in some individuals but in others it was definitely a terminal affair and was not detectable until the eighteenth or twentieth day following insemination. The maximal volume increment varied between 100 and 400 per cent of the average prepregnancy micturition volume.

These tremendous individual differences seemed to be related quite definitely to the outcome of the pregnancy. In the six animals which went through pregnancy and bore litters that lived and were nursed until the time of weaning, the changes in micturition volume during pregnancy were slight and occurred late. Changes in micturition volumes were detectable from the eleventh to the twentieth days and bladder size eventually doubled in some instances but this constituted the maximal change observed in this group. Micturition volume changes occurred earlier, between the fourth and eleventh day of pregnancy, in the five instances in which litters were not nursed and cared for effectively after birth
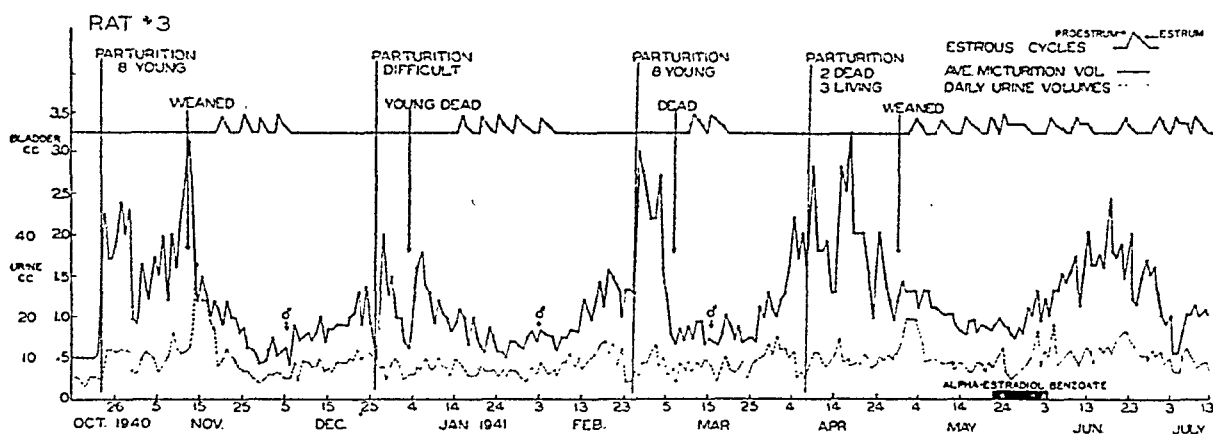
Fig. 3. Rat 3—chart showing effects of pregnancies which terminated normally and abnormally; effects of nursing and weaning, early death of young and injections of alpha-estradiol benzoate (200 rat units daily for 8 days).

(fig. 3). Five additional pregnancies were terminated by late (19 to 20 day) abortions or stillbirths and in these cases the rise in micturition volume had begun by the second or before the seventh day of pregnancy. The micturition volumes attained were three to four hundred per cent greater than the normal volumes for those individuals.

3. *Parturition and period of lactation.* Immediately following parturition there was an enormous increase in micturition volume which frequently attained a level of from two to nine times the normal. The average rise obtained in our series of animals was approximately five hundred per cent. In the group of rats whose litters survived until weaning the maximum micturition volume occurred from one to five days following parturition. These volumes then tended to decrease somewhat but still remained markedly elevated (about 3-fold the normal volume) as long as nursing continued. Within two to nine days following weaning the elevated micturition volume began to subside and reached a normal value in one to two weeks (fig. 3). Concomitant with the elevated micturition volumes there was occasionally a slight increase in the total daily urine output. In these

cases the daily urine output became elevated during the last third of pregnancy and remained elevated until the time of weaning. The total volume during this time was frequently three times the normal. During the first three days following weaning or preweaning-removal of the young there was a temporary rise of the total daily urine volume to a level which averaged five times the normal values (fig. 3). At this time the micturition volume was decreasing. Changes in bladder response consequently do not appear to be directly related to the volume of urine to be eliminated.

Five animals had litters which died within the first week following parturition. As was pointed out previously, a rise in micturition volume had begun rather early in pregnancy (4th to 11th day). In most instances the maximum micturition volume was reached shortly after parturition. The volumes remained elevated as long as the litters lived and usually remained high one or two days after death or removal of the young. The micturition volumes then fell precipitously and reached normal levels within two to ten days. In these animals the rise in total daily urine volumes was less marked than in the previous group and normality was attained immediately after loss of the litter. Comparison of the bladder changes observed to occur in these animals with those occurring when the litters were normally nursed, led us to conclude that lactation or the act of nursing prolonged the period of augmented micturition volume. To test this hypothesis a seven and a ten-day-old litter were removed from their normally lactating and nursing mothers. The two mothers were manifesting the typically increased micturition volumes. There was an immediate decrease in micturition volume and the bladder attained its normal prepregnancy level of response within a few days.

In five animals pregnancies resulted in abortion. The rise in micturition volume had begun very early in pregnancy and reached a peak at the time of abortion. The events, in all cases, were similar to those which occurred in rat 3. In this animal the initial micturition volume was 0.56 cc. Two days following mating the micturition volume began to rise, reaching 1.3 cc. on the 20th day. On the 22nd day of pregnancy the animal began to bleed. At this time the micturition volume rose to 2.2 cc., then dropped rapidly during the next seven days to 0.6 cc. On the seventh day following bleeding the animal aborted a single fetus, following which the volume again rose reaching the maximum value of 1.8 cc. before it began to fall again. This volume was reached on the tenth day. Thirteen days after the fetus was aborted the micturition volumes had returned to normal values. There was practically no rise in total urinary output at any time.

The results obtained from unanesthetized postpartum rats were confirmed by the technique of artificial bladder distention. For example, two nursing female rats were anesthetized and their bladders exposed and artificially filled with warm saline. In both instances repeated tests revealed that the bladder could be filled to a much greater volume than before pregnancy. In one case emptying volumes were 1.84 cc., 2.15 cc., 1.89 cc. as compared to 0.60 cc., 0.38 cc., 0.52 cc. observed before pregnancy and 1.01 cc., 0.79 cc., 0.70 cc. as obtained two weeks after weaning of the young. When exposed to a head of pressure of 90 cm. of

saline the bladder filled at a rate of 0.61 cc. per minute. This rate which was more rapid than normal indicated less tonic resistance to distention. The intravesicular pressure at which micturition occurred averaged 29.2 cm. of water, which is practically identical with the threshold pressure observed in normal rats. The trauma and irritation resulting from exposure and puncture of the viscus evidently caused hyperirritability of the bladder and a greater frequency of micturition. Micturition volumes decreased markedly after the test although they did not immediately attain the normal prepregnancy level.

4. *Injections of alpha-estradiol benzoate.* Twelve female and two male rats were given alpha-estradiol benzoate (Progynon-B, Schering) intramuscularly in amounts ranging from 0.0033 to 0.462 mgm. (20 to 2800 rat units) per rat, these amounts usually being given in divided doses over a period of 1 to 14 days. Total dosages amounted to from 0.1 to 13.9 rat units per gram of body weight. Two to six days following the beginning of such estrogen injections there was a rise in micturition volume similar to that seen in the pregnant animals (fig. 2). In four cases the rise did not begin until one week after termination of the injections. The maximum micturition volumes were attained in from 8 to 19 days after the initial rise became detectable and the peak volumes were from two to four times the normal values. After the maximum volume had been reached there was a progressive drop in the average daily micturition volumes. There was a great deal of variation but the normal characteristic micturition volume was again established within three to nineteen days (figs. 1-B, 3, 4). The two males injected with the estrogenic material showed similar changes though of lesser magnitude. In some instances the rise in micturition volume was accompanied by a two- to five-fold increase in the total daily urine output but there was no apparent relationship between magnitude of increase in fluid elimination and magnitude of bladder emptying volume. The rats varied greatly in their responsiveness to the estrogenic material. Some of the greatest changes in bladder tone resulted from the smaller injections (fig. 4). In two females there was no rise in micturition volume following injection of 0.066 to 0.28 mgm. (400 to 1700 rat units) alpha-estradiol benzoate per rat. It is possibly significant that these same animals likewise failed to show the usual picture of long continued estrus as determined by the vaginal smear method.

Acute experiments performed on six injected rats at the period of greatest micturition volume supported the previously described evidence that this estrogenic material modified bladder responses. The bladder did not contract and the sphincters did not permit escape of fluid until abnormally great volumes were attained. In one instance (fig. 4) the actual figures were 4.03 cc., 3.75 cc. and 3.8 cc. as compared to the animal's previously and subsequently determined normal threshold volumes of 0.83 cc., 0.75 cc. and 0.85 cc. The hypotonic condition permitted more rapid filling of the bladder than occurred before injection though the head of pressure was the same. The filling rate was 0.60 cc. to 1.2 cc. (average 0.88 cc.) per minute. The intravesicular pressure which resulted in opening of the sphincters averaged 27.3 cm. of water which was not greatly different from the average threshold micturition pressure of the normal rats.

5. *Alpha-estradiol benzoate followed by progesterone.* Three animals were given alpha-estradiol benzoate in doses ranging from 0.00079 to 0.0022 mgm. (4.8 to 13.9 R.U.) per gram of body weight, following which they were given progesterone (Proluton, Schering) intramuscularly in dosages ranging from 0.023 to 0.056 mgm. per gram of body weight. The estrogenic material was administered in daily amounts of 200 R.U. per rat for a period of four to fourteen days; the progesterone was given over a period of five to fourteen days, beginning five to twenty-one days after the estrogen injections had started. In one instance a single injection of 200 rat units of estrogenic material was bracketed by injections of progesterone (fig. 4). The observed rises in micturition volume resembled those resulting from the estrogenic material alone. The progesterone as administered did not seem to affect the micturition volume curve which follows injection of alpha-estradiol benzoate. The micturition volume began to rise
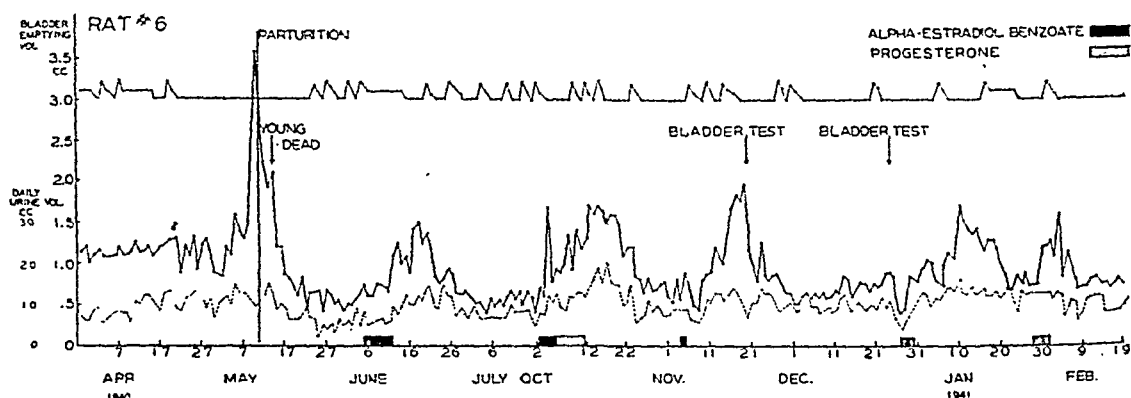
Fig. 4. History of rat 6. First injection—200 rat units daily for 8 days; 2nd injection —200 rat units daily for 4 days followed by daily injections of 1 mgm. of progesterone for 6 days; 3rd injection—20 rat units of estrogenic material for 2 days; 4th injection—1 mgm. progesterone for 5 days. On the third day 20 rat units of estrogenic material also given; 5th injection—2 mgm. of progesterone daily for 4 days.

two to eight days after the beginning of estrogen administration and the rise continued throughout the period of progesterone injection. Maximum micturition volumes of 1.3 cc. to 2.4 cc., representing a 2.2 to 2.8 fold rise in volume, resulted in 8 to 20 days following the onset of the estrogen injections. Recovery occurred and normal micturition volumes were reached between 25 and 33 days after the injections were first started. This showed no clear change from the picture obtained by giving estrogenic material alone (figs. 1-B, 3, 4).

6. *Progesterone.* Eight animals were given progesterone alone in amounts ranging from 4 to 28 mgm. per rat over a period of 4 to 14 days. Two of these rats were males. No definite change in micturition volume resulted. Such quantities of progesterone should have been adequate to affect the uterus and other tissues of the rat (8, 9). These injections did inhibit temporarily the estrous cycles of our animals but neither technique of estimating bladder response showed consistent variations from normal. We were unable to produce by means of this hormonal substance changes in bladder response similar to those observed

to occur in the pregnant and lactating rat. Figure 4 shows a case in which progesterone apparently caused a rise in micturition volume; figure 1-B shows a case in which the hormone seemingly produced a fall. In all other instances no changes were seen.

DISCUSSION. Our measurements of bladder reactions in the rat consistently showed that during the last part of pregnancy and following parturition there is a tremendous rise in micturition volume. During this latter period a level of micturition volume is attained which is approximately five times greater than normal. This elevation is generally maintained following parturition until weaning has occurred. Early death of the young or their early removal also causes a precipitous decrease in micturition volume to the prepregnancy level. That these changes in the volume at which micturition characteristically occurs are due to changes in bladder muscle tone is indicated by several facts. Bladders of anesthetized postpartum rats can be filled artificially to an abnormally large volume before intravesicular pressure rises to a level which causes the sphincters to open. The intravesicular pressure which causes sphincter opening is practically the same in normal as in these atonic bladders. In seeking an explanation one of the most obvious assumptions to make is that the changes in bladder tone have a hormonal origin. Hormones which are known to affect the tone of certain smooth muscles are liberated in great quantity by the placenta during pregnancy and by the ovaries during pregnancy and lactation.

Langworthy, Kolb and Lewis (2) have suggested that progesterone might be the hormonal cause of the observed bladder hypotonia. Corpora lutea develop gradually during pregnancy as do the changes in micturition volume. Corpora lutea are also present during lactation and they degenerate at weaning. Corpora lutea are active during the periods of bladder hypotonia and they degenerate at a time when bladder recovery is occurring. There are, however, several phenomena which cannot be explained on the basis of progesterone production and disappearance. Our failure to reproduce the bladder changes by injections of progesterone blocks acceptance of the theory that this corpus luteum hormone is the sole cause of changes in bladder muscle tone. It might be, however, that some other corpus luteum hormone such as Relaxin is the agent modifying bladder response. We have no evidence either to establish or disprove this latter hypothesis other than the fact that bladder changes similar to those observed in the pregnant and nursing rat can be produced by injections of alpha-estradiol benzoate alone. It is known (9) that from 0.5 to 2.0 mgm. progesterone are required to produce detectable changes in a rat's tissues. The maximum doses which we used were only 14 to 46 times that amount. Estrogen injections, however, were from 20 to 2000 times the minimal effective dosage. It must be admitted that, on this basis of calculation, our progesterone dosages were much smaller in magnitude than were the estrogen injections. Even on this basis of comparison the smaller amounts of estrogenic material which caused bladder changes were less in magnitude than some of the progesterone injections which failed to produce an effect.

It is well known that progesterone alone may have little effect upon tissues but when preceded by estrogens it greatly modifies tissue responses. This applies

to uterine muscle (10).   Uterine and ureteral muscle undergo similar changes during pregnancy (11).   It consequently occurred to us that pretreatment of the rats with estrogenic material might reveal some effect of progesterone on the bladder.   In the majority of experiments, however, progesterone, as given, seemed neither to add to nor detract from the characteristic estrogen effect.

During spontaneously occurring estrus in the cat a marked reduction in micturition volume occurs (4).   Alpha-estradiol benzoate injections produce a similar drop in micturition volume.   Following periods of spontaneous or artificially induced estrus, bladder volumes do tend to rise above normal but never as much as in the rat.   Reactions of the two species during heat and during periods of estrogen injections are quite different.   It may be that neural factors enter in to cause the species difference in bladder response.   The postpartum bladder changes appear to be identical and since estrin seems to have opposite effects on the bladders of the two species this would favor the assumption that corpus luteum hormones rather than estrogens cause bladder hypotonia.   This indirect evidence is not convincing in the light of more direct tests.

Lisco and Biskind (12) have claimed that high concentrations of estrone produce marked changes in the bladder wall and extreme dilatation frequently occurs. In our experiments on the rat injections of alpha-estradiol benzoate also caused a bladder hypotonus.   We never observed a reduction in micturition volume in this species.   Since injections of estrogenic materials produce changes in bladder response similar to those observed to occur following parturition one should consider the possibility that these postpartum changes are due to changes in estrogen production by body tissues.   It is well known that the estrogen content of the urine rises during pregnancy then drops sharply after parturition (13, 14). One peculiarity observed in our experiments was that bladder changes frequently did not appear until after termination of estrogen injections.   In a few rats the estrous cycle had been re-established before the rise in micturition volume had begun.   This might be explained on the basis of a slow action of the hormone on the bladder.   One could also hypothecate that hypotonia occurs most markedly in the bladder as recovery from estrogen modification is taking place.   In the postpartum rat the greatest changes in the bladder occur as estrogen production is diminishing.   The early large increments in micturition volume observed in pregnancies terminating in abortions and abnormal labor might be explained on the basis of abnormal placental function and early termination or diminution of estrogen production.   The observations that nursing or lactation prolongs the period of bladder hypotonus and the sudden termination of suckling causes swift recovery of normal bladder tone are not easily explained on the basis of estrogen production or termination of such a production.   It is possible that the ovaries in addition to producing more corpus luteum hormone during the lactation period are also forming estrogens in considerable quantity.   The fact that estrogens tend to prevent lactation (14) argues against this assumption.   We are not certain that variations in estrogen production are solely responsible for all observed changes in bladder tone.

It is frequently stated that abdominal operations tend to inhibit the bladder.

It has been suggested that the pain and trauma resulting from parturition might cause the postpartum retention. In the rat the changes are of such long duration that this explanation seems inadequate. Furthermore, exposure and puncture of the bladder during artificial perfusion experiments tended to reduce micturition volume (fig. 4). Pain and trauma may modify bladder reflexes but they are not the sole factors.

In some pregnancies bladder changes are rather slight and in some animals modifications of micturition volume do not follow administration of estrogenic material. Despite our lack of full understanding of the origin of changes observed to occur in the rat during pregnancy and following parturition and following hormone injections it seems reasonable to assume that hormones do modify bladder reactions. It is also clear that changes in micturition volume indicate changes in hormone production within the body.

These studies indicate that, at least in the rat, a slight and gradually developing decrease in frequency of micturition during pregnancy is normal. The average volume of individual micturitions may double. A great diminution in frequency and a great (400 to 500 per cent) increase in micturition volumes, however, indicate an abnormal termination of pregnancy.

SUMMARY

In rats there is normally a slight rise in micturition volume during pregnancy and a tremendous rise (averaging over 500 per cent) in the volume of individual micturitions following parturition.

There is a great variation in the rise in micturition volume in rats during pregnancy. This is correlated with the outcome of the pregnancy; i.e., in those animals whose pregnancies end in abortion or in early death of the litter, the rise begins quite early in pregnancy, whereas in those animals that give birth to living litters that survive until the time of weaning, the rise begins relatively late.

Following parturition the micturition volume remains high or is even elevated to a higher level. Micturition volume decreases somewhat during lactation but a normal frequency and volume of micturition is not restored until death of the young or until weaning.

Administration of an estrogenic substance (alpha-estradiol benzoate) produces an elevation of micturition volume which is similar to that seen during pregnancy and following parturition.

Progesterone, as administered in these experiments, does not produce any observable changes in bladder responses in the rat.

REFERENCES

(1) REED, C. B. Am. J. Obstet. 49: 145, 1904.
(2) LANGWORTHY, O. R., L. KOLB AND L. G. LEWIS. Physiology of micturition. Williams & Wilkins Co., Baltimore, 1940.
(3) STANDER, H. J. Williams' Obstetrics. 8th ed., D. Appleton Century Co., New York, 1941.
(4) WOOLSEY, C. N. AND C. McC. BROOKS. This Journal 119: 423, 1937.
(5) LANGWORTHY, O. R. AND C. B. BROCK. Am. J. Obstet. and Gynec. 37: 121, 1939.

(6) LANGWORTHY, O. R., D. L. REEVES AND E. S. TAUBER.   Brain **57**: 266, 1934.

(7) SCHULTZ, W. H.   J. Urol. **34**: 156, 1935.

(8) PHILLIPS, W. A.   This Journal **119**: 623, 1937.

(9) SZARKA, A. J.   J. Endocrinol. **2**: 1, 1940.

(10) WILSON, L. AND R. KURZROK.   Endocrinol. **26**: 587, 1940.

(11) TRAUT, H. F., C. M. McLANE AND A. KUDER.   Surg., Gynec. and Obstet. **64**: 51, 1937.

(12) LISCO, H. AND G. R. BISKIND.   Endocrinol. **27**: 772, 1941.

(13) D'AMOUR, F. E., D. FUNK AND M. B. GLENDENNING.   Proc. Soc. Exper. Biol. and Med. **35**: 26, 1936.

(14) ALLEN, E.   Sex and internal secretions.   2nd ed., Williams & Wilkins Co., Baltimore, 1939.

EFFECTS OF EXTRACTS OF THE HYPOPHYSIS, THE THYROID AND THE ADRENAL CORTEX ON SOME RENAL FUNCTIONS

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We have reported (1) that diodrast[2] (D) and inulin (In) plasma clearances and maximum tubular output of diodrast (DT_m) are markedly decreased by loss of the anterior lobe of the hypophysis. The decreases in D and In plasma clearances were attributed to a decrease in renal blood flow and the decrease in DT_m not only to a decrease in renal blood flow but also to a depression of the functional capacity of the tubules to transport D at high plasma levels. These depressions of renal function were observed as early as 7 days after removal of the glandular hypophysis and they have persisted at the original level or decreased for more than two years. Because of the early onset of the change it was believed that the effect was probably direct rather than indirect through atrophic changes in other endocrine glands.

In this report evidence is presented to support this belief in that it is shown that an extract of the hypophysis of the sheep[2] markedly increases D and In plasma clearances and DT_m in normal, in simple hypophysectomy, in total hypophysectomy and in puncture dogs. Thyroid extract also increases these renal functions but to a lesser degree. Only in the puncture dogs does adrenal cortical extract[3] have any significant incretory effect.

PREPARATION OF ANIMALS. Trained adult female dogs were used as experimental animals. They were on a diet of one pound of raw horse meat daily with Purina dog chow ad lib. Operations on the hypothalamus or hypophysis were carried out through the oral approach. Simple hypophysectomy consisted in the removal of the glandular hypophysis and the posterior lobe. The median eminence was not removed. A dog so prepared does not exhibit diabetes insipidus and does not become markedly obese (0 to 20 per cent increase). Total or complete hypophysectomy consisted in removal of the entire glandular and neural hypophysis (median eminence included). Such a dog exhibits marked diabetes insipidus and becomes quite obese. "Puncture" consisted in the severance of the stalk and the removal with a special instrument of the median eminence and overlying tissues caudal to and above the median eminence. Such a dog exhibits marked diabetes insipidus and becomes markedly obese.

PROCEDURE. Previous to any hormone treatment, control experiments were

performed on all animals to determine D and In plasma clearances and DT_m. After normal values had been obtained, hormone administration was begun and clearance experiments were run, usually on the seventh or eighth and on the fourteenth or fifteenth day after daily hormone administration. Adequate time was allowed for the animals to recover from any of the effects of the hormone therapy before another extract was given. Thyroid extract was given orally, 0.1 gram per kilo per day. In the case of the hypophysectomized animals, all of which showed more or less increase in weight, the dose was given on the basis of their preoperative weights. Such a dose will increase the basal metabolic rate 25 to 40 per cent in 10 to 14 days. Preloban Niphanoid[2] was given subcutaneously, each dog receiving 200 hypophysectomized rat units per day, with the exception of K17 which was given 100 units per day. Adrenal cortical extract[3] was given subcutaneously, some animals receiving 4 cc. per day and others 8 cc.

In the preparation of the animals for carrying out the clearances and the chemical methods for determining urine and plasma inulin and diodrast iodine, the procedures were identical with that described in a previous paper (White, Heinbecker and Rolf, loc. cit.). There were four normal, two simple hypophysectomy, two complete hypophysectomy and two puncture dogs in the series reported on. Of these one dog from each of the groups altered by an operation was autopsied to determine the state of the hypophysis or the hypothalamus. In every instance the examination of serial sections of the region involved indicated the correctness of the label attached to the designated group.

RESULTS. *D plasma clearance.* The results are shown in table 1. Hypophysial extract produces a definite increase in clearance in all dogs tested. The average of three experiments on three normal dogs shows an increase from a base level of 317 cc./min./M^2 to 415 cc.; two experiments on 1 simple hypophysectomy dog, an increase from 124 cc./min./M^2 to 291 cc.; four experiments on two total hypophysectomy dogs, an increase from 179 cc./min./M^2 to 283 cc.; and two experiments on one puncture dog, an increase from 279 cc./min./M^2 to 361 cc. The effect is well established within seven days and the clearance remains elevated with continued administration of the extract up to 15 days. No attempt was made to extend the period of observation.

Thyroid extract produces an increase in clearance in all dogs tested but in general the effect is not so marked as that produced by hypophysial extract. As with hypophysial extract the greatest percentage increase occurred in the simple and total hypophysectomy dogs. In these, renal function was diminished previous to hormone therapy. The average of six experiments on three normal dogs shows an increase from a base level of 362 cc./min./M^2 to only 378 cc.; two experiments on one simple hypophysectomy dog show an increase from 124 cc./min./M^2 to 211 cc.; two experiments on one total hypophysectomy dog, an increase from 153 cc./min./M^2 to 235 cc. and two experiments on one puncture dog, an increase from 279 cc./min./M^2 to only 292 cc.

Adrenal cortical extract either left the clearance unchanged or showed a tendency to decrease it with the exception of the puncture dogs in which there was

an increase. The average of two experiments on two normal dogs showed a variation from a base level of 284 cc./min./M² to 281 cc.; two experiments on two simple hypophysectomy dogs a variation from 116 cc./min./M² to 105 cc.; four experiments on two total hypophysectomy dogs a variation from 179 cc./min./M² to 162 cc. and one experiment on one puncture dog, an increase from 279 cc./min./M² to 324 cc.

In plasma clearance. The results are shown in table 1. Hypophysial extract produces a rise in inulin clearance in all dogs but not to so marked an extent as was observed in the D plasma clearance. The average of three experiments

TABLE 1

	D CLEARANCE			In CLEARANCE			DTm		
	cc./min./M²			*cc./min./M²*			*mg.I/min./M²*		
Normal:									
Before extract..................	317	362	284	95	110	83	21.2	20.8	15
After extract..................	P	T	A	P	T	A	P	T	A
	415	378	281	123	121	107	34.1	30.8	12.3
Simple hypophysectomy:									
Preoperative....................	279	279	273	83	83	79	14.8	14.8	15.2
Postoperative, before extract....	124	124	116	50	50	50	2.4	2.4	2.4
After extract..................	P	T	A	P	T	A	P	T	A
	291	211	105	84	73	47	11.1	11.1	2.2
Total hypophysectomy:									
Preoperative....................	353	344	353	120	119	120	17.8	12.3	17.8
Postoperative, before extract....	179	153	179	68	65	68	6.7	8.8	6.7
After extract..................	P	T	A	P	T	A	P	T	A
	283	235	162	83	71	47	14.4	12.6	5.6
Puncture:									
Preoperative....................				No observations					
Postoperative, before extract....	279	279	279	91	91	91	28.2	23.4	28.2
After extract..................	P	T	A	P	T	A	P	T	A
	361	292	324	131	102	116	34.9	27.6	26.6

P—Anterior pituitary extract; T—Thyroid extract; A—Adrenal cortical extract.

on two normal dogs shows an increase in In plasma clearance from a base level of 95 cc./min./M² to 123 cc.; two experiments on one simple hypophysectomy show an increase from 50 cc./min./M² to 84 cc.; four experiments on two total hypophysectomy dogs show an increase from 68 cc./min./M² to 83 cc. and two experiments on one puncture show an increase from 91 cc./min./M² to 131 cc. Again the effects are quite marked at the end of seven days.

Thyroid extract also produces an increase in In clearance but at the dosages employed the increase is not quite so great as that produced with hypophysial extract. The average of six experiments on three normal dogs shows an increase in In clearance from a base level of 110 cc./min./M² to 121 cc.; two experiments

on one simple show an increase from 50 cc./min./M² to 73 cc.; two experiments on one total show an increase from 65 cc./min./M² to 71 cc. and two experiments on one puncture show an increase from 91 cc./min./M² to 102 cc.

The effects produced by adrenal cortical extract on the In clearance are variable. Both simple and total hypophysectomy dogs showed some tendency toward a decrease, the puncture dogs showed an increase corresponding to the increase in D plasma clearance, thus indicating an actual increase in blood flow. The normal dogs showed an increase in In clearance without a corresponding increase in D plasma clearance, a result probably due to constriction of the

TABLE 2

Puncture dog K 18

DATE	BODY LENGTH	WEIGHT	AVERAGE URINE OUTPUT PER DAY	HORMONE ADMINISTRATION	D CLEAR-ANCE	I CLEAR-ANCE	DTm
	cm.	kgm.	cc.		cc./min./M²	cc./min./M²	mgm.I/min./M²
12/30/40 pre-op.	85.4	14		none	250	82	13.4
10/13/41 237 days post op.		25.7	5,500	none	251	85	13.3
3/ 6/41		31.5	6,000	9 days thyroid extract	388	127	19.95
3/10/42		31		15 days thyroid extract	379	115	34.4
5/14/42		34	6,500	8 days anterior lobe extract	458	128	27.22
5/21/42		34.5		15 days anterior lobe extract	332	116	26.5
7/27/42	91.0	35.5	5,800	8 days adrenal cortical extract, 4 cc./day	340	116	9.95
9/29/42		37	7,000	8 days adrenal cortical extract, 8 cc./day	460	140	21.3
1/ 8/43 686 days post op.		36.2	5,600	none	462	129	18.6
3/26/43	91.0	38	5,500	none	306	100	28.96

glomerular arterioles. The average of two experiments on two normal dogs shows an increase from a base level of 83 cc./min./M² to 107 cc.; two experiments on two simple hypophysectomy dogs show a slight decrease from 50 cc./min./M² to 47 cc.; four experiments on two total hypophysectomized dogs show a decrease from 68 cc./min./M² to 47 cc. and one experiment on one puncture shows an increase from 91 cc./min./M² to 116 cc.

DT_m: The results are shown in table 1. Hypophysial extract produces a very marked increase in DT_m in all animals, a rise greater than that observed in any of the other functions tested. The average of three experiments on three normal dogs showed an increase in DT_m from 21.16 mgm. iodine/min./M² to 34.13 mgm.; two experiments on one simple hypophysectomy show an increase from 2.37

mgm./min./M² to 11.05 mgm.; four experiments on two total hypophysectomy dogs show an increase from 6.71 mgm./min./M² to 14.36 mgm. and one experiment on one puncture showed an increase from 28.17 mgm./min./M² to 34.89 mgm. In all cases in which the DT_m was tested after one week and after two weeks of daily hormone administration, the value at the end of two weeks was higher.

Thyroid extract produced a marked increase in DT_m in the normal, simple hypophysectomy and total hypophysectomy dogs and a small increase in the puncture dogs. The simple hypophysectomy type was the only one showing an increase equal to that produced by a hypophysial extract. The average of six experiments on three normal dogs showed an increase in DT_m from a base level of 20.75 mgm. iodine/min./M² to 30.79 mgm.; two experiments on one simple hypophysectomy an increase from 2.37 mgm./min./M² to 11.10 mgm.; two experiments on one total hypophysectomy an increase from 8.8 mgm./min./M² to 12.57 mgm. and three experiments on two puncture dogs an increase from 23.38 mgm./min./M² to 27.58 mgm.

Adrenal cortical extract left the DT_m almost unchanged with a possible slight tendency toward a decrease. The average of two experiments on two normal dogs showed a change from a base level of 15.02 mgm./min./M² to 12.33 mgm.; one experiment on one simple hypophysectomy a change from 2.37 mgm./min./M² to 2.22 mgm.; four experiments on two total hypophysectomy dogs a change from 6.71 mgm./min./M² to 5.56 mgm. and one experiment on one puncture dog a change from 28.17 mgm./min./M² to 26.6 mgm.

The results on one puncture dog K-18 are presented separately in table 2. They are unique in that the base line for D and In clearances and DT_m increased markedly with time after operation. Interesting also is the observation that this dog even though considered full grown at the time of the operation increased considerably in length postoperatively. It seems possible that in this dog the eosinophil cells of the hypophysis have increased in number or effectiveness over the normal. The results show that hypophysial extract, thyroid extract and adrenal cortical hormone increased certain renal function and renal blood flow but not to the same degree as in the other puncture dog of this series in which the base line remained at its preoperative level.

DISCUSSION. One of the primary objects of this investigation was to test further the validity of the conclusion previously drawn (White, Heinbecker and Rolf, loc. cit.) that the glandular hypophysis exercises a humoral influence on renal blood flow and diodrast transport at high plasma levels. The results herein reported support the thesis in that hypophysial extract has been shown capable of augmenting D and In plasma clearances and DT_m in normal, in simple hypophysectomy, in total hypophysectomy and puncture dogs. Such augmentation is marked where there has been a depression due to the removal of the glandular hypophysis.

Because of the rapidity with which the depression of renal function was initiated after hypophysectomy, it was believed probable that the action of the hypophysial hormone was in large measure a direct one. Among the possible

indirect pathways for such an influence, that of the thyroid and of the adrenal cortex now have been explored. The results indicate that the thyroid gland, particularly in states where thyroid activity is depressed, is capable of augmenting those renal functions which are augmented by hypophysial extract. Certain evidence would indicate that in the intact animal the degree of its influence on the kidney is probably less than that of the glandular hypophysis. Total thyroidectomy depresses the basal metabolic rate at the most 30 to 40 per cent in six to eight weeks. The depression in general blood flow presumably is not greater than this. The 50 per cent or greater decrease in renal blood flow after hypophysectomy comes on within four to six days. Hypophysectomy depresses the basal metabolic rate in dogs 15 to 20 per cent. Again without doubt the depression in general blood flow effected thereby is much below the depression in renal blood flow associated with the loss of the hypophysis.

Additional support for the belief that normally the thyroid gland exercises relatively little influence on the renal functions herein studied or on renal blood flow comes from the finding that in puncture dogs where there is no renal depression regressive changes occur in the thyroid gland similar to those which occur after hypophysectomy. In puncture dogs the changes in the thyroid are secondary to a loss of basophil cells in the anterior lobe of the hypophysis which follows the hypothalamic injury (unpublished data).

The observed effects of cortical extract on renal function in the dog indicate fairly conclusively that the trophic influence of the hypophysis is not exercised through stimulation of the adrenal cortex. Supporting evidence that the adrenal cortex does not influence renal function has been presented by Barnett, Perley and Heinbecker (2) in their studies of a case of Cushing's syndrome due to an adrenal cortical tumor. In this patient D and inulin clearances and DT_m were normal.

Other evidence that the hypophysis exercises a marked influence on the kidney is found in the demonstration by Winternitz et al. (3) and by White, Heinbecker and Rolf (4) that one remaining kidney does not hypertrophy in the hypophysectomized animal. Barnett, Perley and Heinbecker (loc. cit.) also have shown that in acromegaly the urea clearance may be markedly elevated (up to 230 per cent of normal). These investigators hold that under certain conditions renal function studies can serve to measure the functional activity of the eosinophil cells of the glandular hypophysis.

SUMMARY

Subcutaneously administered extracts of the glandular hypophysis increase the diodrast and inulin clearances and the maximum excretion of diodrast at high plasma levels in normal, in simple hypophysectomy, in total hypophysectomy and in "puncture" dogs, i.e., those with a hypothalamic lesion of a type causing diabetes insipidus and obesity.

Orally administered thyroid extract causes a similar though less marked increase in the above renal functions.

Subcutaneously administered adrenal cortical extract does not increase but

tends rather to decrease diodrast clearance in normal, in simple hypophysectomy and in totally hypophysectomized dogs. It increases diodrast clearance in the hypothalamic puncture dog. Inulin clearance in normal, in simple and in total hypophysectomy dogs usually is decreased by it but occasionally there is a moderate increase believed due to constriction of the glomerular arterioles. It increases diodrast and inulin clearances in the hypothalamic puncture dog. Diodrast T_m is decreased in the normal and in all the types of experimental dogs studied.

The results are interpreted as indicating that in normal, in simple and in total hypophysectomized dogs anterior lobe extract and thyroid extract increase renal blood flow and also increase the capacity of the renal tubules to transport diodrast at high plasma levels. Adrenal cortical extract does not increase, probably decreases renal blood flow slightly. It does not increase the capacity of the renal tubules to transport diodrast at high plasma levels.

In dogs of the "puncture" group anterior lobe extract, thyroid and adrenal cortical extract increase renal blood flow. Anterior lobe and thyroid extracts increase the capacity of the renal tubules to transport diodrast at high plasma levels but adrenal cortical extract does not.

The great effectiveness of anterior lobe extract in increasing renal blood flow and the capacity of renal tubules to transport diodrast is support for earlier evidence indicating that the anterior lobe of the hypophysis exerts a trophic influence on the kidney.

REFERENCES

(1) WHITE, H. L., P. HEINBECKER AND D. ROLF. This Journal **136**: 584, 1942.
(2) BARNETT, H. L., A. M. PERLEY AND P. HEINBECKER. Proc. Soc. Exper. Biol. and Med. **52**: 114, 1943.
(3) WINTERNITZ, M. D. AND L. L. WATERS. Yale J. Biol. and Med. **12**: 705, 1940.
(4) WHITE, H. L., P. HEINBECKER AND D. ROLF. Proc. Soc. Exper. Biol. and Med. **46**: 44, 1941.

SLOWING OF THE HEART AS A CONDITIONED REFLEX IN THE RABBIT

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Reflex slowing of the heart in the human subject is demonstrable in response to pressure on the eyeball—the oculocardiac reflex—and to inhalation of irritating vapors which stimulate either the endings of the nasal branches of the 5th nerve, or the sensory endings of the pulmonary vagus. The oculocardiac reflex has been made the subject of successful conditioning in man by eliciting this reflex at the same time as sounding a bell (1). Since the rabbit exhibits so marked a cardio-inhibitory response to inhalation of ammonia, it was thought that this animal might prove to be a good subject for determing whether slowing of the heart could be conditioned in a lower animal as well as in man.

A special canvas sling was prepared to support the rabbit with its legs hanging down through holes. Straps over its back held the animal in a comfortable position and prevented too violent struggling. Zinc electrodes on the shaven legs which had been moistened with saline, made connection with a string galvanometer. The animal was so placed that it could not see the experimenter as he manipulated the galvanometer, and the room was quiet and in darkness except for the light necessary to watch and to record movements of the shadow of the galvanometer string. A tube from a bottle containing ammonium hydroxide terminated just beneath the rabbit's nose. By means of a foot lever the operator could let loose a stream of ammonia vapor from this tube, the vapor being forced out by air which entered the bottle under pressure.

Each rabbit was brought into the experimental room at the same time each day and was handled very gently to avoid frightening. In accordance with Pavlov's suggestion an animal was allowed to become accustomed to the presence of one experimenter, and the same person handled it throughout the entire procedure.

Twenty minutes' rest was allowed after placing the animal in position, since it was found that this period was sufficient for the animal to become relaxed and the heart rate steady, an absolutely necessary condition for the experiment. This could readily be established by counting the swings of the shadow of the galvanometer string visible to the operator. This preliminary procedure was repeated on three successive days in order to accustom the animal to the experimental setup. Photographic records were then taken to show the normal heart rate, the effect of the bell alone, and of the ammonia alone. The heart rate was determined as beats per second using the crest of the QRS complex as guides and making measurements with as great an accuracy as possible by means of dividers. The film was run at a rate of 4 cm. per sec., with a time marker indicating each $\frac{1}{25}$ of a sec.

Conditioning was begun by sounding the bell and releasing the ammonia simultaneously. In a preliminary experiment in which a stethoscope was used to count the heart beats, the bell was rung for 20 sec. and at the same time an open ammonia bottle was thrust under the animal's nose. After 50 such pairings there appeared to be a decrease of about 50 beats per min. to the sound of the bell without application of ammonia. The difficulty in recording accurately a heart rate of over 200 beats per min. by this method led to the use of the electrocardiograph, but the time of ringing the bell and the amount of ammonia inhaled had to be reduced in order to avoid bodily movements which interfered with the electrical recording of the heart beat. A further experiment in which the bell was rung for 1 sec. only gave slight but definite indication of conditioning. In the final experiments the bell was rung for 5 sec. and the ammonia was allowed to flow only until the shadow of the galvanometer string showed that the animal's heart rate had become definitely slower, a matter of about 3 sec. Five such pairings were made daily with a rest of at least 5 min. between trials. To test whether conditioning had been established, the bell was rung without releasing ammonia at the end of each 10 pairings. Conditioning was evident after 20 pairings, but the response after 30 was much more striking.

Seven young but fully grown rabbits were put through this routine, three females and four males. Two of them, both males, proved unmanageable. Their movements so interfered with the recording that even after 20 trials they had to be discarded. The results on each of the five rabbits which we were able to condition were essentially the same, whether the animals were male or female.

The normal heart beat, once the animal had settled down in the cradle, showed a slight degree of arrythmia, but in the quiet, darkened room the greatest variation from the mean for minutes at a time would be 12 beats per min., usually less. Average rates were 228, 234, 222, 217, each characteristic of a given rabbit on a given day. The effect of the bell alone at the beginning of experimentation was an immediate increase in heart rate. This was evident from watching the shadow of the galvanometer string, but was impossible to count on the photographic records because of the irregularities due to general muscular movements in this "startle" response. The effect of inhalation of a single whiff of ammonia at the beginning of experimentation was an immediate and striking slowing of the heart rate from some 225 beats to about 55 per min. The slow rhythm persisted for about 7 sec. when the rate began to pick up. It had returned to normal within another 15–20 sec.

After conditioning was established the response to the bell alone was as follows: an immediate irregularity of the heart beat with indications of an increase of 12–18 beats per min.; in less than 2 sec. this was followed by an abrupt decrease of at least 30–36 beats per min. each with a plainly marked QRS complex. This rate was 18–24 beats below the normal resting rate and therefore beyond the range of the normal arrythmia. The effect is perhaps more strikingly shown by timing the intervals between individual beats as in the following record of rabbit G:

Before the bell was sounded the interval was 0.26 sec. for 4 consecutive beats. Immediately following the first stroke of the bell the interval shortened to 0.16 sec., and then

apparently to 0.10, the 2nd QRS complex being difficult to make out. The 3rd interval lengthened to 0.28, the 4th to 0.33, and throughout the rest of the 5 sec. period while the bell was ringing the interval remained in the neighborhood of 0.37. The interval returned to 0.26 exactly 3.6 sec. after the bell stopped ringing and remained at approximately this figure. On the basis of beats per min. this means that the rate before and after the bell was 230 per min. while during the ringing of the bell it was 162, a decrease of 68 beats per min.

It must be emphasized that intervals between beats when ammonia was presented might be as long as 1.3 sec., whereas in the conditioned response the interval was never more than 0.4 sec. Nevertheless the time by which the interval lengthened during ringing of the bell alone after 30 pairings with ammonia, viz., 0.11 sec. in the protocol quoted above, was more than 5 times the maximum variation observed while the animal was at rest, viz., 0.02 sec., the latter, of course, being either a lengthening or shortening of the interval. This, therefore, was considered sufficient evidence that conditioning had been established.

Further proof that this was indeed a case of conditioning was afforded by use of the method of extinction. Each rabbit was at the end of the experiment exposed to the sound of the bell alone without ammonia for 20 trials. Electrocardiographic records taken at the end of this period showed only the short preliminary increase in heart rate or no change at all. There was no decrease in rate during the 5 sec. of bell ringing beyond a return to the normal rhythm with its possible variation of 12 beats per min. The conditioned slowing of the heart had become extinct.

SUMMARY

It is possible so to condition a rabbit as to slow its heart rate by using inhalation of ammonia as the unconditioned stimulus and the ringing of a bell as the conditioned stimulus.

REFERENCE

(1) SAROKHTIN, J., K. U. TURGEL AND O. MINUT-SAROKHTINA. Zh. Fiziol. U.S.S.R. 17: 207, 1934.

DISTRIBUTION OF CORTICAL POTENTIALS RESULTING FROM INSUFFLATION OF VAPORS INTO THE NOSTRILS AND FROM STIMULATION OF THE OLFACTORY BULBS AND THE PYRIFORM LOBE[1]

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The purpose of this study was to determine by oscillographic procedure the distribution of the afferent association fibers from the pyriform lobe of the dog to other parts of the cerebral cortex, and to record briefly some confirmatory work on the afferent fibers from the olfactory bulb to the pyriform lobe.

Anatomical connections from the olfactory bulb to the pyriform lobe and amygdaloid nuclei have been established by many investigators. Hasama (1934) obtained action potential spikes from the pyriform lobe as a result of inhalations of quajacol, indol and chinolin. These were not obtained after cocainization of the nasal epithelium. Adrian (1942) obtained strong potential waves from inhalation and insufflations of air into the nostrils and these waves were altered when the air included clove and asafetida vapors. From this he concluded that the olfactory organ could be stimulated mechanically and chemically. Magoun *et al.* have reported by abstract that potentials were obtained from the pyriform lobe of the cat following stimulation of the olfactory bulb. The writer modified somewhat the technique of Hasama and Adrian. Xylol, a more powerful olfactory stimulant than cloves and asafetida (1929), was used in addition, and mechanical stimulations through the trigeminal and vagus nerves were eliminated by a previous procedure (1928–9). The approximate time of stimulation of the nasal epithelium by a vapor was recorded in the middle tracing of each record (fig. 2, A) by vapor from a branched tube contacting a microphone.

Oscillograph records from the pyriform lobe (fig. 1, points *2, 3* and *4*) taken at the time of insufflation of xylol, cloves, asafetida or room air always demonstrated conspicuous spikes within approximately one-fifth of a second from the time the vapor reached the nasal epithelium (fig. 2 A, top record, middle for microphone and bottom for time). Likewise single shock stimulation of the olfactory bulbs was followed in approximately 10 milliseconds by pronounced spikes (fig. 2 B). Ordinarily the spikes following bulb stimulation were the largest and those from the air insufflations were the smallest.

Stimulation of the *pyriform lobe* at points *3* or *4* (fig. 1) with single shock resulted in conspicuous spikes in approximately 3 milliseconds from the ventrolateral portion of the prefrontal area (fig. 1, points *8, 9, 10* and possibly *20*), but not from other cortical areas (points *5* to *7* and *11* to *19*). Stimulation of the pyriform lobe was followed by antidromic potentials in the olfactory bulb, but

stimulation of the bulb did not alter the rhythm from points *8, 9* and *10* from the prefrontal area (fig. 2. D). After under cutting the prefrontal area at points *8, 9* and *10* from the rear in one dog, stimulation of the pyriform lobe no longer elicited spikes from the prefrontal area.
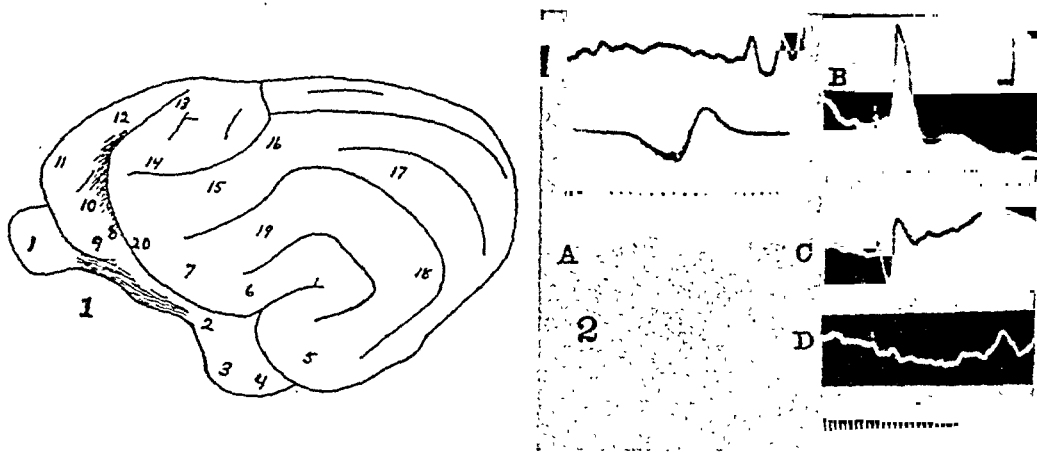
Fig. 1. Areas indicated by numerals represent points on a dog's brain which were stimulated or from which potentials were recorded.

Fig. 2. Oscillograph records in which an upward spike is negative. A. Top row, from pyriform lobe at time asafetida was insufflated into the nostril; middle row, simultaneous microphone record starting approximately at the time the vapor reached the olfactory epithelium; bottom row, 60 cps. 0.3 mv. B. From pyriform lobe during single shock stimulation of olfactory bulb. C. From prefrontal area (8) during stimulation of pyriform lobe. D. From prefrontal area (8) during stimulation of olfactory bulb. Records B to D, 200 cps. 0.3 mv.

DISCUSSION. While Adrian's deduction may be correct that the olfactory organ is stimulated mechanically from air insufflations as well as from olfactory stimulating vapors, it is also possible that the keen olfactory sense of dogs may detect odors from inhalations or insufflations of air that cannot be detected by man. The spikes following our air insufflations were not elicited from trigeminal or vagal stimulation.

That potentials were recorded from the dog's prefrontal cortex (points *8, 9* and *10*) following stimulation of the pyriform lobe is not surprising since it suggests the presence of association fibers corresponding to the uncinate fasciculus of higher vertebrates. The presence of association fibers from the pyriform lobe to the prefrontal area may explain the significance of these areas for obtaining correct conditioned differential foreleg responses with olfactory analysers (1940, 1941) and the relative unimportance of the prefrontal area for obtaining correct differential responses with auditory, general cutaneous and optic stimuli (1943).

SUMMARY

Action potentials from the pyriform lobe followed single shock stimulation of the olfactory bulbs or insufflations of xylol, cloves, asafetida or laboratory air into the nostrils of dogs in which trigeminal and vagal stimulation had been eliminated.

Single shock stimulation of the pyriform lobe evoked potentials from the ventro-lateral portion of the prefrontal area and antidromically from the olfactory bulbs, but not from other cortical areas. No spikes were recorded from the prefrontal area following stimulation of the olfactory bulbs or from stimulating the pyriform lobe of one dog after areas *8*, *9* and *10* had been under cut from the rear.

REFERENCES

ADRIAN, E. D. J. Physiol. **100**: 459, 1942.
ALLEN, W. F. This Journal **87**: 319, 558, 1928–9.
       Ibid. **88**: 117, 1929.
       Ibid. **128**: 754, 1940.
       Ibid. **132**: 81, 1941.
       Ibid. **139**: 525, 1943.
HASAMA, B. Pflüger's Arch. **234**: 748, 1934.
MAGOUN ET AL. Abstract in Anat. Rec. **85**: 327, 1943.

THE INFLUENCE OF THE LIVER ON THE PROTEINS OF THE BLOOD PLASMA

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The association of disorders of the liver with the diminution of the total protein of the plasma was shown in clinical studies of the blood in hepatic disease by Grenet (1) and by Gilbert and Chiray (2). Numerous subsequent investigations have confirmed and extended these observations so that the liver appears to be a definite factor in the regulation of the protein content of the plasma. In the experimental animal Mann and Magath (3) found that after total removal of the liver there were a decrease of the concentration of albumin in the plasma and a tendency for the reversal of the albumin-globulin ratio. Similar observations have also been reported by Cantô (4) and by Franke, Toczyski and Lankosz (5).

Various other experimental conditions affecting the liver have been employed in studies of the plasma proteins. Kerr, Hurwitz and Whipple (6) found that the protein content of plasma of animals depleted by plasmapheresis was rapidly replaced by the formation of new plasma protein and that the globulin portion was replaced more rapidly than the albumin. Injury to the liver produced by the administration of chloroform or phosphorus resulted in marked delay of the regeneration of the plasma proteins lost by plasmapheresis. The extent of the delay of regeneration depended on the extent of the hepatic injury and in many experiments the time of the return of the plasma proteins to their normal level was coincident with the completion of repair of the injured hepatic tissue. The amount and character of the diet were also found to influence the curve of the regeneration of plasma protein. Jürgens and Gebhardt (7) found an increase of the globulin of the plasma of Eck fistula dogs within two to three weeks after operation. *Feeding of meat decreased the amount of albumin and increased the globulin present,* and carbohydrate diets tended to maintain a more normal ratio. Sawada (8) found a decrease of the concentration of albumin and a slight increase of that of globulin of animals that had experienced hepatic damage following poisoning by phosphorus, chloroform or carbon tetrachloride as well as after inoculation with Schistosoma japonicum. In the hepatic damage following ligation of the common bile duct Bollman (9), Sawada (8) and Henriques and Klausen (10) found that the total protein of the plasma was decreased one to three months after operation. The albumin was markedly reduced and the globulin tended to be increased.

Similar reductions of the concentration of the serum protein have been observed in certain types of renal disease and in various types of malnutrition. That the fundamental cause of the alteration of the serum protein is not adequately explained either by the loss of protein from the body or by its inadequate intake has been discussed by Melnick and Cowgill (11). These authors indi-

cated that there is a failure of some mechanism of formation of plasma protein as well as the excessive loss or diminished intake of protein. By diet alone Holman, Mahoney and Whipple (12) caused alterations of the plasma proteins similar to those associated with diseases of the liver. Madden, Winslow, Howland and Whipple (13) found that formation of plasma protein may be greatly depressed in the presence of bacterial infection and inflammation. Under the influence of local inflammatory processes such as are produced by the subcutaneous injection of turpentine the regeneration of plasma proteins was only about a third as efficient as in the same animal with similar diets before and after production of the abscess. During this period the nitrogen balance was not altered to give indication of increased destruction of protein. Madden and Whipple (14) have published an excellent review of studies of the source, production and utilization of the plasma proteins.

In our studies we have determined the changes that occur in the protein content of the plasma after complete removal of the livers of dogs. The concentration of proteins of the plasma was reduced by plasmapheresis after hepatectomy and the influx of protein from the tissues other than the liver was determined by the subsequent alteration of the plasma proteins. Similar observations were made on normal dogs, and on dogs after procedures by which alterations of hepatic function were produced.

METHODS. The animals used in these experiments were maintained on a standard diet consisting of 38 per cent ground lean horse meat, 35 per cent cracker meal, 15 per cent cooked tomatoes, 8 per cent lard and 4 per cent bone ash. This was fed once daily in amounts equivalent to twice the calculated basal caloric requirement of each animal. Variation of the protein content of the diet was accomplished by changing the meat content of the foregoing diet. Plasmapheresis was accomplished by withdrawal of blood equivalent to a fifth to a third of the estimated blood volume with subsequent reinjection of the erythrocytes. The blood was taken into a fifth volume of 2.5 per cent solution of sodium citrate, of which only traces remained after centrifugation and withdrawal of the plasma. The erythrocytes were washed once with saline solution and resuspended in saline solution prior to reinjection. When larger amounts of plasma were to be removed, additional bleeding and reinjections were repeated in the same manner. The total amount of serum protein removed was calculated from aliquot portions of each bleeding.

Removal of the liver was accomplished by means of the three-stage method. After operation the animals recovered from the ether anesthesia and were in good condition when plasmapheresis was begun one hour after completion of the operation. In some experiments blood was withdrawn and reinjections were made by direct puncture of an artery or a vein and in some experiments cannulation of the femoral artery was employed, procaine hydrochloride being used as a local anesthetic agent in the region involved. The concentration of sugar in the blood was maintained within normal limits by the continuous intravenous injection of 10 per cent solution of glucose.

The total protein, fibrinogen, euglobulin, pseudoglobulin and albumin of the

plasma were determined by Howe's modification of Kjeldahl's method, in which the nitrogen of the fractions is determined after precipitation within zones of increasing concentrations of sodium sulfate.

The determination of plasma volume, using the blue azo dye T 1824, has been made whenever possible, and the calculation has been made from this figure with the hematocrit value and the protein content of the plasma. Subsequent calculations assumed that the volume of circulating cells remained unchanged and the total blood volume was calculated on this basis. Other studies indicate that the volume of circulating cells as determined by the hematocrit varies with physiologic conditions. Because of increased cellular stasis after plasmapheresis, the calculated plasma volume is probably subject to considerable error and circulatory changes at different times may vary the extent of this error. Such errors, however, do not seem sufficient to alter the general trend of the results reported.

RESULTS. Regeneration of plasma protein was studied after plasmapheresis of a third of the calculated blood volume under a number of different circumstances. The observed animals included normal animals, animals immediately after splenectomy, after removal of a third to a half of the liver, animals after alteration of the blood supply to the liver (Eck's fistula), and animals that had varying degrees of experimental hepatitis produced by repeated inhalation of carbon tetrachloride vapor. In most series, plasmapheresis was done after a preliminary feeding period of a week or more of three different diets. One diet consisted of lean meat, to another carbohydrate was added so the diet contained 12 per cent of protein and the third diet contained but 1 per cent of protein.

In all of the foregoing control experiments calculation of the data obtained after plasmapheresis indicated that some protein was added to the plasma during the first hour. Most of the addition was globulin in amounts up to approximately 1 gm. for each kilogram of body weight, and measurable amounts of albumin were also added. The greatest amounts of protein were added in the dogs which had received diets containing 12 per cent of protein, somewhat less was added in dogs receiving only meat and only 0.2 or 0.3 gm. of protein was added in animals that had received diets containing 1 per cent of protein. The Eck fistula animals and those that had extreme hepatic damage from carbon tetrachloride added only 0.1 to 0.2 gm. of protein for each kilogram of body weight and previous diet did not definitely influence this amount. Six to nine hours after plasmapheresis fibrinogen had returned to its original level or more in all animals except those that had severely damaged livers and the Eck fistula animals. Small amounts of globulin were added in all animals. Very small additions of albumin were detectable in dogs which had received diets containing 12 or 20 per cent protein except in the Eck fistula animals or those that had severe hepatic damage.

The data from which the foregoing conclusions were drawn were obtained immediately after ether anesthesia from animals which received dextrose intravenously during the period of experiment. The primary purpose of the experiments was to simulate the conditions which were employed in the animals after complete removal of the liver. The changes of the amount of circulating proteins

were calculated from the amount of plasma protein removed and from the total volume of plasma as determined at various intervals in the different experiments by the dye method. The main uncertainty of the calculations is the possibility that the sample of venous blood taken for analysis did not represent a true sample of entire blood of the animal. Stasis of blood in the capillaries probably occurs frequently in the experimental animals. While our results were consistent, it should be pointed out that the changes found were small. More ex-

TABLE 1

Plasma proteins after total removal of the liver

HOURS AFTER HEPATECTOMY	HEMATOCRIT	TOTAL PROTEIN	PER CENT OF TOTAL PROTEIN PRESENT AS			
			Albumin	Pseudoglobulin	Euglobulin	Fibrinogen
	per cent cells	*grams per 100 cc.*				
0	51.5	7.54	38.4	45.2	10.2	6.24
6	48.8	6.55	38.4	49.2	10.2	2.29
18	49.2	6.19	35.2	51.8	11.5	1.02
0	45.0	6.47	67.7	16.1	13.1	3.25
6	41.8	5.84	59.2	25.8	13.6	1.37
12	46.0	5.48	57.7	27.6	14.2	0.55
18	42.7	5.22	56.4	31.4	12.1	0.20
0	53.0	7.11	41.0	46.5	9.3	3.2
6	44.2	5.34	40.7	53.1	3.2	3.1
14	41.3	4.56	43.7	53.2	2.2	1.1
0	40.0	7.34	31.8	49.8	9.95	8.59
6	31.8	5.77	33.8	53.8	7.45	5.37
12	29.0	5.32	39.9	51.1	4.89	4.14
18	24.8	5.00	35.8	57.2	1.60	5.40
24	19.6	4.37	35.0	61.1	0	3.90
1	51.2	7.06	61.5	24.6	12.2	1.5
7	42.4	5.05	62.0	31.3	6.8	0
13	39.0	4.75	63.7	29.1	7.2	0
24	34.8	4.30	63.8	27.5	8.8	0
27	38.7	4.19	63.3	25.6	9.6	1.6
30	28.0	3.31	68.4	24.8	6.4	0.9

tensive plasmapheresis would be desirable, but we were unable to accomplish it in the dog after complete removal of the liver.

The effect of total removal of the liver. Because the greatest changes were found in the control animals receiving a diet containing 12 per cent of protein, all of the animals received this diet for at least seven days prior to total removal of the liver. The results of five typical experiments are given in table 1.

In all of these experiments some dilution of the proteins of the plasma occurred. In most cases the dilution of the proteins of the plasma was of the same order as

the dilution of the erythrocytes. This would suggest that the plasma protein was lost as whole blood, although the amount of blood in the peritoneal cavity at necropsy was not sufficient to account for this loss. Recalculation of the data so that the loss of plasma was proportionate to the loss of erythrocytes indicated that there was a loss (or dilution) of total plasma protein of 5 to 10 per cent. The loss of albumin was also 5 to 10 per cent while the loss of fibrinogen was 30 to 100 per cent. The loss of euglobulin was from 3 to 100 per cent in different experiments. The values for pseudoglobulin were usually increased, either a slight increase or as much as 66 per cent more than the original value. All of these changes occurred within the first few hours after operation. Subsequent analyses of specimens obtained up to thirty hours after removal of the liver showed only minor variations, but no major changes of protein distribution. Determinations of blood volume also indicated no great change of blood volume during this period.

The results of these experiments indicate that during the period of readjustment immediately following removal of the liver there may be an addition of pseudoglobulin to the blood. In no instance in these experiments did the addition amount to more than 0.5 gram for each kilogram of body weight of the animal. There is a somewhat irregular destruction or loss of fibrinogen and euglobulin but no appreciable change of the albumin of the plasma. In subsequent periods up to thirty hours there is no indication of loss or addition of proteins of the plasma.

Plasmapheresis after hepatectomy. In different experiments a third to a half of the total blood volume was withdrawn from the carotid artery of the dehepatized animal within a period of ten to twenty minutes. On completion of the bleeding an equivalent amount of washed erythrocytes from normal dogs was rapidly injected into the same artery. The erythrocytes were suspended in saline solution to the same volume as the blood withdrawn. Analyses were made on the first blood withdrawn. The animals were maintained by the continuous intravenous injection of 10 per cent solution of glucose in amounts sufficient to maintain normal values for blood sugar.

The results of two typical experiments of this series are given in table 2. In contrast to the hemodilution usually found in dogs maintained by continuous infusion of glucose after removal of the liver, hemoconcentration occurred following plasmapheresis. It is apparent that little protein is added to the blood of the dehepatized animal after plasmapheresis and that the colloidal osmotic pressure of the plasma is maintained by hemoconcentration. Calculation of the data on the assumption that concentration of the plasma has occurred equal to the concentration of erythrocytes indicates that some increase of the protein content of the plasma occurred during the procedure of plasmapheresis. In these experiments from 0.5 to 0.7 gram of globulin for each 100 cc. of plasma appeared to have been added during the fifteen or twenty minute procedure. The albumin content of the plasma was found to be very close to that expected from the withdrawal of the amount of blood and the dilution of the remaining blood with the saline suspension of erythrocytes. During subsequent periods, up to

nine hours after plasmapheresis, only minor changes occurred in the protein content of the plasma. There was usually a slight loss of protein from the plasma, which was probably due to a small loss of whole blood and subsequent dilution of the circulating plasma. In no case did there appear to be an appreciable destruction of albumin nor was there any replacement of this substance after total removal of the liver and plasmapheresis. In all experiments there was no loss of globulin and in a few there was a very slight increase of the globulin of the plasma several hours after plasmapheresis.

COMMENT. Some of the difficulties in the investigation of the effect of removal of the liver on formation of plasma protein have been mentioned. The necessary anesthesia, surgical procedures, loss of blood and administration of dextrose have some effect on the circulating blood. The samples of blood withdrawn from the larger vessels represent a true sample of the mixed circulating blood but give small indication of the state of the blood in the capillaries, which may be more

TABLE 2

Plasmapheresis after total removal of the liver

HOURS AFTER HEPAT-ECTOMY	HEMATOCRIT	PLASMA PROTEIN	ALBUMIN	GLOBULIN	
	per cent	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	
1	31.3	7.80	5.40	2.40	Withdrew 40 cc. of blood for each
1¼	34.8	6.00	3.96	2.04	kilogram of body weight and injected
10¼	44.7	7.00	4.50	2.50	an equivalent amount of erythrocytes in saline solution
1	51.2	6.90	5.30	1.60	Withdrew 50 cc. of blood for each kilo-
1⅓	58.0	4.50	3.10	1.40	gram of body weight and injected an
7⅓	83.2	6.60	4.40	2.20	equivalent amount of erythrocytes in saline solution

concentrated or more dilute than the sample taken for analysis. This fact makes the interpretation of sudden changes hazardous, especially at the time of plasmapheresis. The calculation of the addition of protein to the plasma immediately associated with plasmapheresis is based on the increased proportion of globulin in the plasma beyond that which was withdrawn and at times an increase of globulin to more than the original level.

The fact that the changes due to plasmapheresis are present for several hours suggests that the observations are not due to a temporary change of concentration of the blood due to capillary stasis. The immediate influx of protein into the plasma during plasmapheresis may be due, however, at least in part, to the imposed alterations on the circulatory system as well as to the withdrawal of protein. Similar changes occur during the operation for complete removal of the liver. The only animals that did not add appreciable quantities of globulin to their plasma during plasmapheresis were Eck fistula animals, animals that had severely damaged livers and animals on diets deficient in protein. In all of these

the plasma albumin was definitely subnormal before plasmapheresis was done. Plasmapheresis after complete removal of the liver causes the addition of globulin to the plasma similar to that which occurs in normal animals.

Many of the questions concerning the rôle of the liver in the production of plasma proteins remain unanswerable. Many of the changes that occur in the plasma proteins coincident with removal of the liver are obviously due to causes other than the absence of the liver, since there is no consistent loss or gain with the progression of time following the operation. It would appear that in the absence of the liver the loss of protein from the plasma is too small to be measured by our methods within periods up to thirty hours after the removal of the liver. Plasmapheresis experiments after complete removal of the liver failed to indicate any regeneration of plasma protein within periods which were adequate for the demonstration of some regeneration of plasma protein in normal control animals.

SUMMARY

During the period of circulatory readjustment immediately following complete removal of the liver some globulin appears to be added to the plasma. Somewhat similar amounts of globulin are added to the plasma of well fed control dogs during circulatory readjustments associated with plasmapheresis. The addition of globulin is diminished or absent in animals that have Eck fistulas or severely damaged livers when the protein content of the plasma was previously abnormal. The immediate changes of concentration of the plasma proteins after complete removal of the liver are a small loss, or dilution, of total protein with a similar change of the albumin content, a marked loss of fibrinogen and euglobulin and an increase of the pseudoglobulin of the plasma. In subsequent periods up to thirty hours after removal of the liver there is little evidence of loss or addition of protein to the plasma. In periods up to nine hours after plasmapheresis in the liverless animal there is no evidence of regeneration of plasma proteins.

REFERENCES

(1) GRENET, H. Compt. rend. Soc. de Biol. 63: 552, 1907.
(2) GILBERT, H. AND M. CHIRAY. Compt. rend. Soc. de Biol. 63: 487, 1907.
(3) MANN, F. C. AND T. B. MAGATH. Ergebn. d. Physiol. 23: 212, 1924.
(4) CANTÓ, A. Compt. rend. Soc. de Biol. 104: 1103, 1930.
(5) FRANKE, M., T. TOCZYSKI AND J. LANKOSZ. Compt. rend. Soc. de Biol. 119: 1209, 1935.
(6) KERR, W. J., S. H. HURWITZ AND G. H. WHIPPLE. This Journal 47: 356, 370, 379, 1918.
(7) JÜRGENS, R. AND F. GEBHARDT. Arch. f. Exper. Path. u. Pharmakol. 174: 532, 1934.
(8) SAWADA, T. Japan. J. Gastroenterol. 3: 38, 1931.
(9) BOLLMAN, J. L. Proc. Staff Meet., Mayo Clin. 3: 137, 1928.
(10) HENRIQUES, V. AND U. KLAUSEN. Biochem. Ztschr. 254: 414, 1932.
(11) MELNICK, D. AND G. R. COWGILL. Yale J. Biol. and Med. 10: 49, 1937.
(12) HOLMAN, R. L., E. B. MAHONEY AND G. H. WHIPPLE. J. Exper. Med. 59: 251, 1934.
(13) MADDEN, S. C., P. M. WINSLOW, J. W. HOWLAND AND G. H. WHIPPLE. J. Exper. Med. 65: 431, 1937.
(14) MADDEN, S. C. AND G. H. WHIPPLE. Physiol. Rev. 20: 194, 1940.

THE EFFECT OF FASTING AND DEHYDRATION ON THE BLOOD SUGAR RESPONSE TO INJECTED INSULIN IN THE CHICK[1]

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It has been reported recently (1) that the chick offers possibilities as a test animal for the assay of insulin. Contrary to the general impression, the blood glucose of the chick is lowered in response to light doses of insulin and the duration of the hypoglycemia is shortened by increasing the dose. The percentage decrease of blood glucose $1\frac{1}{2}$ hours after injection is found to be a straight line function of the logarithm of the dose up to about 1.5 units per kilogram. Although the individual variation in response is not excessive, attempts have been made to reduce this variation by prolonged fasting and dehydration. Some success has been achieved in this direction and, in addition, some facts concerning the effects of long fasting and dehydration on blood sugar and insulin sensitivity have been observed.

METHODS. Single comb White Leghorn cockerels were used throughout this investigation. They were received one day after hatching and placed in an electrically heated battery brooder and provided with Wayne Chick Starter mash and water at all times. The chicks were used between 30 and 40 days of age at which time the body weight varied between 200 and 400 grams. The average weight of each of the series was between 242 and 305 grams. Blood glucose was determined by the Hagedorn-Jensen method on 0.1 cc. of blood obtained from the wing vein (2). The dilution of the unmodified insulin[2] was made with acidified distilled water, pH 2.4, in such a way that the volume injected was always 0.5 cc.

The experimental animals are divided into two categories: group A consists of four series, each series being fasted 14, 24, 48 or 72 hours; group B contains three series, each series being deprived of both food and water for periods of 24, 48 or 72 hours.

Control blood sugars were taken in the afternoon following the fast, or fast plus dehydration, and the chicks immediately weighed and injected intramuscularly with 0.1875 unit of insulin. A second sample was drawn one and one-half hours after the injection. The same chick was never used more than once.

The nature of the results demand some measure of statistical analysis. Since the number of chicks varies from series to series, the significance of comparisons between series was tested by the expression: $M_1 - M_2/\sqrt{\sigma_{m_1}^2 + \sigma_{m_2}^2} = t$. The comparisons of variance were made by a modification of Fisher's Z test using the table of variance ratios (F) given by Snedecor (3). The values of F for the 5 per cent level of significance are given along with the data in table 2.

A total of 207 chicks was used in this study.

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[1] Contribution no. 319 from the Department of Zoology.

[2] The insulin (Iletin) was supplied by Eli Lilly Co., Indianapolis, Indiana.

A. Control blood glucose and insulin sensitivity. *Results and dis-cussion.* An inspection of table 1 shows that the blood sugars of both groups rose after prolonged fasting. In group A the control blood sugar increases significantly from 144 mgm. per cent after 14 hours' fast to 154 mgm. per cent after 24 hours of fast. Another significant rise occurs in this group between 48 and 72 hours of treatment. The 24 hour control value of 162 mgm. per cent found in group B, the fasted and dehydrated group, is significantly higher than the comparable control value of group A and the rise continues through the 48 and 72 hour series. The difference between the 24 and 48 hour series control blood sugars of group B is highly significant, but the difference between the 48 and 72 hour series falls short of significance. It is clear that the concentration of blood sugar increases both during fasting alone and with fasting plus dehydration. Dehydration would seem to have an augmentative effect and, at the

TABLE 1

The effects of fasting and dehydration on the blood glucose and insulin sensitivity of 30–40 day old chicks

TREATMENT	NO. OF CHICKS	CONTROL BLOOD SUGAR	1½ HOURS AFTER 0.1875 UNIT OF INSULIN	PER CENT CHANGE IN BLOOD SUGAR	AVER-AGE WEIGHT
Group A					
					grams
14 hour fast....................	30	144 ±2.1	118 ±2.4	−18.0 ±1.92	256
24 hour fast....................	31	154 ±2.0	128 ±3.9	−16.9 ±1.78	286
48 hour fast....................	30	159 ±2.4	125 ±3.0	−21.4 ±1.75	269
72 hour fast....................	19	175 ±3.3	140 ±3.4	−20.0 ±1.66	305
Group B					
24 hour fast plus dehydration...	38	162 ±1.8	136 ±2.8	−16.0 ±1.41	242
48 hour fast plus dehydration...	29	179 ±2.0	139 ±2.1	−22.3 ±1.31	244
72 hour fast plus dehydration...	30	184 ±1.9	154 ±2.8	−16.3 ±1.25	248

same time, cause a marked rise between 24 and 48 hours of treatment whereas, with fasting alone, this marked rise does not occur until after 48 hours of fasting.

The blood sugar response to injected insulin parallels the rise in control blood sugar very closely with one exception. The 48 hour series of group B had an initial blood sugar value of 179 mgm. per cent which fell to 139 mgm. per cent after insulin while the 72 hour series had only a slightly higher initial blood sugar but the drop in response to insulin was significantly less than that obtained in the 48 hour series. Upon comparing these two series it would appear that an insulin resistance had developed.

It is customary in mammalian work to express insulin sensitivity in terms of percentage change in blood sugar. The results of this investigation are also expressed in this fashion (see table 1), but since the duration of effect decreases with an increasing dose in the chick, no account can be taken of the time factor and the blood sugar level at 1½ hours after injection is used arbitrarily as the

measure of insulin sensitivity. These figures are very interesting. Apparent marked increases in sensitivity occur in the 48 hour series of each group and the 72 hour series of group A would appear to be more sensitive than the 72 hour series of group B. The greatest increase in response is in group B where the sensitivity changes from −16.0 per cent at 24 hours to −22.3 per cent at 48 hours. A similar rise is noted in the comparable series of group A. Both of these differences are significant. Again, comparing the 72 hour series of groups A and B, the 72 hour series of group A responds with a decrease of 20.0 per cent while the decrease of the 72 hour series of group B is only 16.3 per cent. This difference in sensitivity is probably significant, if account is taken of the considerable difference in body weights of the two groups (19 per cent). Unpublished data show that heavier chicks are slightly less sensitive to insulin than the lighter birds (4).

However, it cannot be stated with certainty that the fasted and dehydrated chicks are less sensitive after 72 hours of treatment than those which were only fasted. If the 14 hour series is taken as a reference point, then good proportionality exists between the control blood sugar level and the level after insulin injection of all series in both groups A and B. The greatest deviation from the expected value occurs in the 48 hour series of each group. In group A the expected value was 130 mgm. per cent, but a value of 125 mgm. per cent was observed; in group B 146 mgm. per cent was expected and 139 mgm. per cent found. However, these differences are slight and probably not significant, but it is interesting that they occur at a time when the control blood sugar is rising rapidly or on the verge of doing so. The blood sugar levels after insulin may be calculated in the same way for the 72 hour series of groups A and B. They are 143 and 150 mgm. per cent respectively. Using these figures in calculating the percentage change gives values of approximately −18 per cent for each series. Therefore, no significant difference would exist. The same is true in the other cases of apparent increased or decreased sensitivity.

An increase of blood glucose after 48 to 96 hours of fasting has been observed both in fowl and mammals (5, 6, 7). Selye reported (7) that rats are resistant to toxic doses of insulin after 48 hours' fast and also found a fasting hyperglycemia. These results are ascribed to an increased secretion by the adrenal cortex which would raise the blood sugar level and confer a non-specific resistance upon the animals, the "alarm reaction." Perhaps it would not be wise to press the comparison of chicks and rats too far, but some observations can be made. From the results presented here, it cannot be said that chicks exhibit an insulin resistance after fasting and in this respect they would seem to differ from the rat. The blood sugar of the fasting chick rises after 14 hours of fast and continues to do so for the duration of the experiment (72 hrs.). Again this is contrary to the finding on the rat under similar conditions where the blood sugar continues to decline until after 48 hours of fasting. If the prompt rise observed in the chick is to be explained entirely as a result of an "alarm reaction," then we must say that the development of this reaction occurs with considerable speed and that the extent of the reaction is increased considerably by dehydration.

An increase of blood sugar during fasting raises the question of the effect of fasting and/or dehydration on the utilization of carbohydrate. Evidence from mammalian sources (8) indicates that intact fasting animals do not readily utilize carbohydrate. There is also good evidence that the oxidation of carbohydrate is not impaired by fasting alone and the difficulty in disposing of administered glucose is due to the continued glucose formation from protein by the liver (9). Further, Golden and Long (10) have reported that the adrenal cortical extracts will raise the blood sugar and liver glycogen of the chicks as in the mammals. Considering these facts, a mechanism can be visualized whereby the chick can resist the hypoglycemic effects of prolonged fasting and insulin and may succeed in overcoming them to some extent. One might deduce that either the production rate of carbohydrate increases or the utilization rate decreases progressively after about the 14th hour of fasting. In either case, a rise in blood sugar occurs. The effect of this mechanism is such that sufficient carbohydrate is available to replace that which disappears after insulin injection and to prevent an increased sensitivity after prolonged fasting.

B. COMPARISON OF VARIABILITY OF RESPONSE. *Results and discussion.* The comparison of variance between experimental groups has two purposes: first, for the purpose of biological assay, it is desirable to use the least variable material providing that it is sufficiently sensitive, and second, the changes in variance under different treatment undoubtedly have a physiological significance and a knowledge of such changes may give a clue to the mechanism involved. Table 2 shows the comparisons made between the mean square deviations of groups A and B and between series within the same group. The variance in control blood sugar levels and levels after insulin injection must be considered, because the variance of the calculated percentage change in blood sugar presumably is a combination of these variances.

No significant variation is observed in the control blood sugar values of the three series of the fasted group, but there is a tendency toward greater variability with increasing length of fast. The chicks of group B, fasted and dehydrated, have perhaps a little less variation initially than those of group A and the variation tends to decrease with length of treatment. The divergence of the two groups is such that the difference in variance after 72 hours of treatment is of doubtful significance.

The behavior of the variance of the blood sugar values after insulin injection is rather peculiar. In Group A the variation within a series is least after 14 hours of fast, $s^2 = 171$, and the greatest after 24 hours when $s^2 = 470$. Thereafter the variance becomes progressively less. The ratio between the 14 and 24 hour series is highly significant. This is not true of group B where the *least* variance occurs after 48 hours without food or water. The variance of the 48 hour series as compared to the 24 hour series is significantly less (2.20) and that of the 72 hour series falls just short of being significantly greater than that of the 48 hour series. Upon comparing the two groups, A and B, it is apparent that only the 48 hour series are divergent enough to give a significant result.

The only point at which the variance of the calculated percentage change in

blood sugar is significant is at 48 hours.   It is interesting to note that, in these data, the figures on the significance of the variance follows the value of the F ratio for the blood sugar values after insulin and not the F ratio of the control blood sugar comparisons.

TABLE 2

Comparisons of variability in blood glucose of fasted and fasted and dehydrated chicks before and after insulin

COMPARISONS BETWEEN	NO. OF CHICKS	CONTROL BLOOD GLUCOSE		BLOOD GLUCOSE AFTER INSULIN		PERCENTAGE CHANGE		VALUE OF F FOR 5% SIGNIFICANCE
		Variance (s^2)	F ratio	Variance (s^2)	F ratio	Variance (s^2)	F ratio	
24 hr. series								
Group A............	31	144	1.12	470	1.59	100	1.28	1.76
Group B............	38	235		295		78		
48 hr. series								
Group A............	30	169	1.35	265	1.98	91	1.87	1.85
Group B............	29	125		134		49		
72 hr. series								
Group A............	19	214	1.93	223	1.06	52	1.16	2.07
Group B............	30	111		237		60		
Group A								
14 hr. series.........	30	130	1.11	171	2.74	116	1.16	1.84
24 hr. series.........	31	144		470		100		
Group A								
24 hr. series.........	31	144	1.48	470	2.11	100	1.93	2.07
72 hr. series.........	19	214		223		52		
Group B								
24 hr. series.........	38			295	2.20	78	1.59	1.80
48 hr. series.........	29			134		49		
Group B								
48 hr. series.........	29			134	1.77			1.85
72 hr. series.........	30			237				

$$\text{Variance } (s^2) = \frac{\Sigma d^2}{n-1}.$$

The reason for the fluctuations of the variance under different treatment is not clear, but the trends indicate that the homeostatic mechanism is certainly disrupted in some cases and aided in others.   There is reason to believe (1) that the stored carbohydrate in the liver is somewhat higher after 72 hours of fast than after 14 or 24 hours of such treatment.   This may account for the increasing variability of the fasted group.   Such an explanation, of course, must presume that carbohydrate is being manufactured from some endogenous source.

However, the blood sugar of the fasted and dehydrated group is higher than that of the fasted only group, yet the variability is markedly less. Unfortunately, no figures on stored carbohydrates for this group are available.

While no direct evidence can be offered at this time, it is suggested that these changes in variance represent changes in the fuel and operating economy of the chick. Once the stored carbohydrate levels have reached a minimum, protein, or fat, or both must be converted to carbohydrate in order to maintain the essential reactions involving this substance. Undoubtedly the differences in variance between the fasted and the fasted and dehydrated chicks have a physiological significance which remains for further elucidation.

SUMMARY AND CONCLUSIONS

1. Thirty to 40-day old chicks were subjected to fasting, or fasting plus dehydration, for periods of 14 to 72 hours and their sensitivity to 0.1875 unit of insulin was tested.

2. The control blood sugar rose after the 14th hour and continued to rise through 72 hours of fasting. Dehydration augments this rise. The blood sugar values of the fasted and dehydrated chicks rose more promptly and to higher levels than those of chicks which were only fasted.

3. No significant increase or decrease in sensitivity to insulin was observed in either group, although the 72 hour fasted and dehydrated group tended to be less sensitive than the 72 hour fasted group. It is concluded that neither fasting alone nor fasting plus dehydration affects the insulin sensitivity of the chick.

4. Fasting plus dehydration results in a marked decrease in the variability of blood sugar values. Fasting alone tends to increase the variability of control figures. The variance in blood sugar values $1\frac{1}{2}$ hours after insulin injection is least in the chicks fasted and dehydrated for 48 hours.

5. The precision of the results after 14 hours of fast seems sufficient for routine insulin assay by the chick method.

REFERENCES

(1) OPDYKE, D. F. Endocrinology **31**: 363, 1942.
(2) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Williams & Wilkins, 1932.
(3) SNEDECOR, G. W. Statistical methods. Iowa State College Press, 1940.
(4) OPDYKE, D. F. Unpublished data.
(5) MACOWAN, M. M. AND H. E. MAGEE. Quart. J. Exper. Physiol. **21**: 275, 1931.
(6) HENRY, K. M., A. J. MACDONALD, AND H. E. MAGEE. J. Exper. Biol. **10**: 153, 1933.
(7) SELYE, H. Endocrinology **27**: 434, 1940.
(8) DANN, M. AND W. H. CHAMBERS. J. Biol. Chem. **89**: 675, 1930.
(9) SOSKIN, S. AND I. A. MIRSKY. This Journal **114**: 106, 1935–36.
(10) GOLDEN, W. R. C. AND C. N. H. LONG. Endocrinology **30**: 675, 1942.

THE RELATION OF PHYSICAL EXERTION TO THE RESISTANCE OF RED BLOOD CELLS TO LAKING

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For a number of years it has been observed in this laboratory (1) that when a small amount of blood was introduced into distilled water, as in the first step of the volumetric method for the determination of lactic acid employed here (2), the resulting mixture showed various degrees of opacity. The opacity was greater when blood was drawn from subjects who had been running on a treadmill, and there seemed to be a rough relation between the degree of opacity and the severity of the exercise and the amount of lactic acid in the blood. Microscopic examination of the sediment after centrifugation revealed numerous red blood cells and shells of partially laked red blood cells. The unhemolyzed red blood cells which could be counted numbered between 500 and 1,000 per cu. mm. of the original blood. The introduction of plasma alone into distilled water did not produce opacity to a significant degree.

An empirical method of estimating the degrees of opacity was developed in order to study the phenomenon further and to compare the findings with the amount of muscular exercise and with lactic acid determination of the blood.

METHOD. Distilled water is brought to pH 6.8 by the addition of $M/15$ Na_2HOP_4. (In this laboratory, the distilled water requires about 1 to 1.5 cc. of $M/15$ Na_2HPO_4 per liter.) Brom-thymol-blue is used as an indicator (light blue color) employing Sorenson's Na_2HPO_4 and KH_2PO_4 buffers for control. The adjustment of pH is facilitated by employing the photo-electric colorimeter, filter no. 540.)

It is convenient to use capillary blood (collected by finger puncture) although venous and arterial blood give similar results; 0.2 cc. of blood is added to 10 cc. of the buffered distilled water in a colorimeter tube. Care should be taken not to bubble air into the solution while making the transfer. The contents are mixed gently. A drop of caprylic alcohol may be added if necessary. The mixture is allowed to stand 10 minutes before reading. It is probably inadvisable to allow the mixture to stand more than one hour.

The Evelyn photoelectric colorimeter with filter no. 660 has been used. With a tube containing 10 cc. of water the galvanometer thread is set at 100. The reading without the tube of water in place is noted. The blood mixture tube is inserted and a reading is made (G_1). One drop of NH_4OH is added, which clears the mixture. The tube is agitated gently, and a second reading is made immediately (G_2). The density values ($L = 2 - \log G$) for the corresponding galvanometer readings are obtained. The difference between L_1 and L_2 represents the "opacity" of the blood mixture. For example:

	G_1	L_1	G_2	L_2	$L_1 - L_2$ ("OPACITY")
1. Subject at rest.....................	84	0.0757	90_1	0.0446	0.031
2. Same subject after run (2 minutes, 7 m. per hr., grade 8.6 per cent)....	80_1	0.0955	89_2	0.0482	0.047

The increase of opacity of the blood-water mixture after exercise is associated with reduced pH values of the plasma. When blood is equilibrated in a tonometer with different concentrations of CO_2 the opacity increases with the concentration of CO_2. The same is true when small amounts of lactic acid and of HCL are added to the blood. Alkalinization of the blood results in reduced degrees of opacity. When CO_2 or the breath is blown into the water before the blood is added to it, or when the pH of the water is reduced, for example, by addition of small amounts of KH_2PO_4 or of acetic acid, the opacity of the blood-water mixture is increased to a degree roughly parallel to the acidity of the water.

It must be emphasized that the resistance of a small percentage of the red blood cells to laking by water is not necessarily related to the total span of resistance of the majority of red blood cells to hypotonic salt solutions. Indeed, in vitro experiments show that rendering the blood more acid *reduces* the resistance of the *majority* of red blood cells to hypotonic salt solutions. This has been shown previously by Ham (3). It is less clear what happens to the bulk of the red blood cells in this respect in man after exercise. Hastings found that in dogs after exercise the resistance of the red blood cells was increased (4).

Chemical changes of the blood during strenuous exercise influence in some manner the resistance of the red blood cells to laking. Thus, a rough parallelism has been shown to exist between the opacity of the blood-water mixture and both the blood lactic acid and the degree of exhaustion after a run. This is illustrated in tables 1 and 2. When the speed of the treadmill was increased the opacity values increased in every case. When the blood lactic acid was about 50 mgm. per 100 cc. the opacity range was approximately from 0.024 to 0.035. When the lactic acid was about 100 mgm., the opacity range was approximately 0.028 to 0.045. When the lactic acid was about 140 mgm. the opacity range was approximately from 0.045 to 0.072. There was an inverse relation between the physical fitness index and both the lactic acid and the opacity reading. In the untrained men, the opacity increased with the duration of the run and was greater in younger individuals.

Other changes of the blood in addition to the lactic acid undoubtedly influence the resistance of the red blood cells. In the untrained men the opacity on the whole was less for given values of lactic acid than in the trained men. In another series of eight individuals exercising on the treadmill there was close relation between the maximum pulse during exercise (taken with a cardiotachometer) and the opacity of the blood taken 5 minutes after exercise, whereas the lactic acid values did not show a relation to maximum pulse in this group of subjects. In the larger series reported here, both opacity and lactic acid

were roughly related to the pulse rate one minute *after* exercise, the lactic acid-pulse relation being the closer.

TABLE 1

Blood "opacity" readings and other data in college cross country runners (ages 17–21)

Blood samples were taken 5 minutes after a run on a motor-driven treadmill. Cases are arranged in the first part of the table according to the degree of "opacity." In the second part of the table are shown the results obtained on the same subjects after a faster run.

CASE NO.	TREADMILL SPEED: 7 M.P.H., GRADE 8.6%				TREADMILL SPEED: 8.6 M.P.H., GRADE 8.6%			
	Duration of run	Physical fitness index*	Lactic acid	"Opacity" ($L_1 - L_2$)	Duration of run	Physical fitness index*	Lactic acid	"Opacity" ($L_1 - L_2$)
	seconds		*mgm./100 cc.*		*seconds*		*mgm./100 cc.*	
1	300	99	48	0.024	300	106	113	0.043
2	300	106	26	0.026	300	112	108	0.044
3	300	91	28	0.028	300	97	96	0.040
4	300	98	50	0.028	300	103	116	0.043
5	300	80	48	0.030	300	116	89	0.043
6	300	96	48	0.030				
7	300	78	79	0.030	192	64		0.064
8	300	86	50	0.031	300	107	119	0.049
9	300	85	49	0.034	300	97	169	0.075
10	300	92	50	0.034	300	98	119	0.050
11	300	95	52	0.034				
12	300	85	85	0.037	167	58	121	0.065
13	300	88	60	0.037				
14	300	83	65	0.038	215	76	113	0.049
15	300	75	99	0.042	195	57	180	0.079
16	300	79	107	0.042				
17	300	82	92	0.043	217	68	118	0.055
18	300	81	124	0.048				
19					274	88	126	0.062
20					300	101	146	0.065
21					265	73	157	0.065
22					300	98	145	0.073
Average...	300	87.7	64.4	0.0342	266	89.3	127.1	0.0567

* The physical fitness index is obtained by dividing the duration of the run in seconds ($\times$ 100) by twice the sum of pulses 1–1½, 2–2½ and 4–4½ minutes after the run (5). In the second part of the table, physical fitness index was multiplied by 1.26 in order to make the index comparable to that obtained when the speed of the treadmill was 7 miles per hour.

The test for opacity as described has proved in this laboratory to be a simple and brief method for judging the degree of exhaustion and in a manner the determination and grit of young men placed in conditions requiring severe exertion. It will assist in judging the physical fitness of men. Further study of the factors of the blood in exercise, responsible for the changes in resistance

of red blood cells, should add to knowledge of the physico-chemical equilibria of the body.

TABLE 2

Blood "opacity" readings and other data in miscellaneous group of men, out of condition
(ages 25–57)

Blood samples were taken 5 minutes after a run on a motor-driven treadmill (7 m. per. hr., grade 8.6%). Cases are arranged according to degree of "opacity."

CASE NO.	AGES	DURATION OF RUN	PHYSICAL FITNESS INDEX*	LACTIC ACID	"OPACITY" ($L_1 - L_2$)
		seconds		*mgm./100 cc.*	
23	57	62	18	83	0.026
24	40	82	26	95	0.028
25	40	92	25	106	0.029
26	38	85	23	88	0.033
27	30	75	18	100	0.033
28	37	67	17	105	0.033
29	35	89	25	111	0.033
30	45	85	23	112	0.033
31	32	63	18	62	0.035
32	48	77	24	103	0.036
33	31	67	16	113	0.038
34	31	73	18	103	0.039
35	33	76	18	117	0.041
36	26	94	24	131	0.041
37	35	53	13	93	0.042
38	42	68	18	87	0.043
39	32	77	17	128	0.043
40	30	122	28	129	0.043
41	43	70	17	117	0.044
42	34	22	5		0.045
43	34	145	39	133	0.047
44	42	135	30	137	0.047
45	38	87	18	138	0.047
46	31	155	42	108	0.049
47	25	185	47	157	0.053
48	26	100	24	133	0.056
49	28	163	40	135	0.056
Average	35	91.4	23.4	112.4	0.0404

* See footnote to table 1.

SUMMARY

A small percentage of circulating red blood cells is resistant to laking by water. The opacity which results when a small amount of blood is added to a large amount of water can be measured by a simple method employing the photoelectric colorimeter. This opacity increases after exercise, is dependent on the severity of the exercise, and is roughly proportional to the lactic acid of the blood. The opacity increases when blood is exposed in vitro to various

acids.   Undoubtedly factors in addition to the lactic acid concentration which change in vivo with exercise, influence the resistance of the red blood cells.

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REFERENCES

(1) THOMPSON, J. W. AND R. E. JOHNSON.   Personal communications.
(2) EDWARDS, H. T.   J. Biol. Chem. **125:** 571, 1938.
(3) HAM, T. H.   Arch. Int. Med. **64:** 1271, 1939.
(4) HASTINGS, A. B.   U. S. Public Health Bull., No. 117, May, 1921.
(5) JOHNSON, R. E., L. BROUHA AND R. C. DARLING.   Revue canadienne de Biol. **1:** 491, 1942.

THE VASOPRESSOR EFFECT OF THERMAL TRAUMA[1]

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In recent experiments on gastric secretion and motility in burns (1) it was observed that the blood pressure frequently rose during the burn and often remained for some time at or above the control level. This was contrary to our experience with the changes in blood pressure following traumatization (2). A review of the literature revealed that, although many others had reported similar observations, no extensive analysis of this phenomenon has been made. A preliminary report on the causes of the elevation of blood pressure following burns has been presented previously (3), and this paper is the complete report and an extension of the work.

Simultaneously with our report (3), Kabat and Hedin attributed the rise in blood pressure during burns to a nervous factor (4, 5). They found that, following thermal trauma to normal anesthetized cats, an immediate and sustained rise in blood pressure occurred, while in similar experiments with spinal cats a temporary fall in blood pressure was followed by a lesser rise. Similar observations had been made by others. In 1901, in a monograph on his extensive work, Crile (6) described the effect on blood pressure of a nervous factor in burns. He prevented the rise of blood pressure following burns of the hindlegs of cats by injecting cocain into the subarachnoid space of the lumbar spinal cord or by blocking the afferent nerves. In 1931 a rise in blood pressure following burns in dogs was reported by Johnson and Blalock (7) who stated: "During the time that heat was being applied, the blood pressure usually fell slightly. This was not always the case as in some instances it definitely rose." Harkins (8), in 1935, reported that in two of three burn experiments in dogs a rise in blood pressure occurred, and in his monograph (9) he cited similar results from a number of authors. In a detailed study of burns, Lambret and Driessens (11) in 1937 performed experiments on spinal cats similar to those of Kabat and Hedin (4, 5). They believe that the rise in blood pressure during the burn was due to a sudden discharge of epinephrin. This view is supported by the earlier findings of Hartman et al. (12) in 1926, that the concentration of epinephrin in the blood of cats is increased following burns. The rise in blood pressure after burns was also reported in humans by Wilson (10) in 1938. He states: "Forty cases—were admitted within 2 hours after injury; in 35, and amongst these were some of the most extensive and severe injuries, the systolic level of blood-pressure was normal or, in a few, actually above normal."

Our preliminary work (3) convinced us that the pressor effect of burns was

not due to any single factor. We, therefore, have performed 125 experiments on dogs and cats in an attempt to analyze this phenomenon further.

EXPERIMENTAL PROCEDURE. Normal, healthy, fasting dogs and cats were used. All animals were kept under deep anesthesia with sodium pentobarbital (nembutal), diallylbarbituric acid (dial), sodium isoamylethylbarbiturate (sodium amytal), sodium barbital and morphine or ether. Only acute experiments were performed, at the termination of which an excess of anesthetic was administered. Blood pressure was recorded from the carotid artery with a mercury manometer. Third degree burns were administered with boiling water or with a torch. The duration and extent of the burns varied in different experiments. Circulating time was determined by the sodium cyanide method as described previously (13). Hematocrit values were obtained with Wintrobe tubes centrifuged at 2000 r.p.m. for 30 minutes. Arterial plasma CO_2 was determined by the mano-metric method of Van Slyke and Neill and plasma proteins by the falling drop method (14).

TABLE 1

Effect of burns on blood pressure

GROUP NO.	SPECIES	TOTAL NO.	PROCEDURE	AVERAGE BLOOD PRESS. CHANGE $\pm$
				mm. Hg
1	Dog	26	Scalds or burns	+50
2	Dog	2	Burns, const. inj. A.C.*	+75
3	Dog	4	Hypophysis exposed, burn	+50
4	Dog	3	Hypophysectomy, burn	−45
5	Cat	15	Foot scalded	+60
6	Cat	7	Denervated foot scalded	−20

* Acetylcholine iodide, constant intravenous injection.

RESULTS. In table 1 six groups of experiments are summarized. Following extensive torch burns or scalding, twenty-six dogs (group 1) showed an average rise in blood pressure of 50 mm. Hg. Figure 1 represents one typical experiment; the columns of data demonstrate blood changes. The data in the left column are controls, in the middle column, one hour after the first scald, and in the right column, one hour after a second scald. The immediate result of the first (severe) scald was a sharp rise of the blood pressure which remained elevated for 15 minutes. One hour after the burn, the blood pressure was at approximately the control level. The effect of the second scald on blood pressure was slight. One hour later the blood pressure still had remained at a normal level, but the animal was in shock, as indicated by the other determinations. Considerable hemoconcentration, acidosis and slow circulation were present, and the animal died shortly. Six similar experiments with blood studies were performed; the average changes one hour after a single scald were as follows: a slight rise in blood pressure, a 10 second increase in the circulating time, a hemoconcentration of 24 per cent, and a 13 volume per cent in arterial plasma CO_2.

The above studies were continued on the cat which seemed to give a greater pressor response to burns than the dog (group 5, table 1). After some experimentation as to extent, type and degree of burn necessary to give fairly constant results, we found that immersing one foot up to the ankle in boiling water for 30 seconds gave the most consistent results. The effect was an immediate sharp rise of blood pressure, which was maintained in many experiments for a considerable period of time. As shown in table 1, group 5, the average rise of blood pressure in 16 cats was 60 mm. Hg. In a typical experiment, represented in figure 2, the blood pressure was still elevated 35 mm. Hg above the control level fifty minutes after scalding. At that time, the hematocrit value had increased from 36 to 47 per cent indicating a peripheral red cell concentration of 31 per cent above the control level. One and one-half hours after the burn, the blood
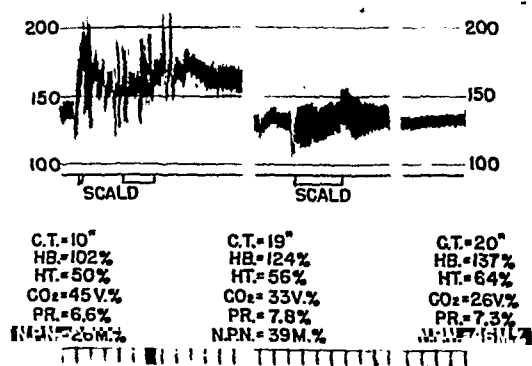
Fig. 1
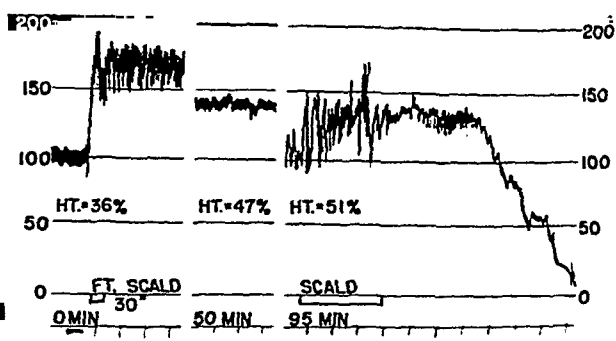
Fig. 2

Fig. 1. Normal dog, male, 18 kgm. Nembutal. Blood pressure tracing during and after a severe scald. Time in minutes. *C.T.* = circulating time; *HB.* = hemoglobin; *HT.* = hematocrit; CO_2 = arterial plasma CO_2; *PR.* = plasma proteins; *N.P.N.* = non protein nitrogen.

Fig. 2. Normal cat, female, 3 kgm. Nembutal. Blood pressure tracing during and after scalding.

pressure had returned to the control level. However, seven minutes after an additional scald this animal died, although the blood pressure was well maintained until a few minutes before death.

In order to explore the possibility of a nervous factor in burn shock, experiments were performed on cats in which one leg was denervated by section of the sciatic and femoral nerves. The central end of the cut sciatic nerve was stimulated with faradic current to simulate an increase in the number of afferent impulses from a burned area. Figure 3 represents a typical experiment. When the nerve was stimulated with alternating induced current (first arrow, fig. 3) there occurred a sharp rise in the blood pressure similar to that observed after a burn. With the cessation of the stimulation (second arrow) the blood pressure immediately dropped to slightly below the control level. This response differed somewhat from the usual response to a first burn, but after a burn pain impulses may continue to arise for some time. After the blood pressure had returned to the control level, the denervated foot was scalded for 30 seconds, and the blood pressure did not rise during the burn but dropped slightly.

After a short interval, the intact foot was scalded. The blood pressure rise was marked but only of short duration. This transitory rise in blood pressure occurred in many experiments; that is, a second burn rarely would have as striking a result as the first one. The pressor phenomenon was more constant and was usually greater and of longer duration with the first burn. The depression of blood pressure, following the scalding of a denervated limb, was confirmed in seven experiments, in which the average drop of blood pressure was 20 mm. Hg. These experiments are listed in group 6 of table 1.

The abolition of the typical pressor response by deafferentation of the limb before scalding suggested a reflex mechanism, possibly through the splanchnic nerves. Therefore, a series of experiments was performed on acutely adrenalectomized cats and on splanchnectomized dogs. Only such animals were used, which had a more or less normal blood pressure following the operation. The
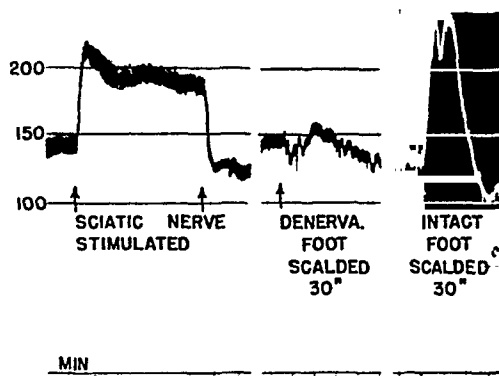
Fig. 3

Fig. 4

Fig. 3. Cat, female, 4 kgm., denervated leg. Nembutal. Blood pressure tracing; femoral and sciatic nerves sectioned on one side.
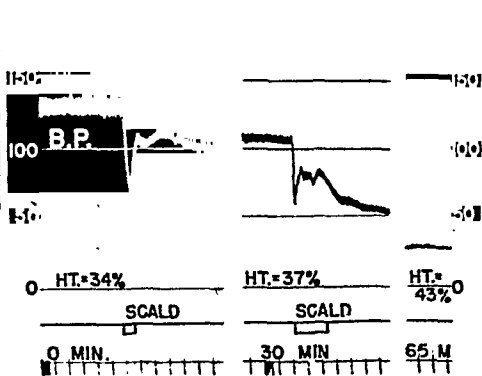
Fig. 4. Dog, male, 16 kgm., hypophysectomized. Nembutal. Blood pressure tracing.

blood pressure rose immediately when the animals were burned, but in the adrenalectomy experiments the initial rise was less and the duration of the rise was shorter than in the controls.

The role of the spleen as a significant factor in the vasopressor effect of burns was investigated on acutely splenectomized dogs. The vasopressor response to a scald was not abolished by splenectomy.

The experiments on adrenalectomized and splanchnectomized animals had shown that the adrenal medulla and the splanchnic nerves played only a minor rôle if any in the reflex rise of blood pressure following burns. Therefore other mechanisms had to be considered. Lim and associates (15) and Necheles and Neuwelt (16) have shown that pituitary pressor hormone can be liberated reflexly by stimulation of afferent nerves. Therefore the next series of experiments were performed on dogs, hyphophysectomized by the temporal approach. One hour after the operation, the blood pressure of the animals usually had returned to near control levels and the first scald was administered; in all three experiments performed, blood pressure dropped immediately. The average drop in blood pressure was 45 mm. Hg (table 1, group 4). This drop was similar to that

seen following mechanical traumatization (17). Four control experiments (table 1, group 3) were done in which the hypophysis was exposed, but not removed. Blood pressures in these sham experiments were at or near control levels at the time of the burn, and the pressor response to a burn was similar to that of the normal animal (table 1, group 1).

The experiments with exclusion of the hypophysis seemed to prove that the reflex rise of blood pressure following a burn was of a neuro-humoral nature. However, the absence of the secretion of a pressor hormone of this gland might have changed capillary tone in such a way that we were working with a different biological test object. In order to avoid this objection, we attempted to detect an increase of pituitary pressor hormone following burns in dogs with intact hypophyses.

In 1938 Necheles and Neuwelt (16) described a sensitive biologic test for pitressin. They found that small doses of pitressin or of pituitrin injected intravenously, inhibited the vasodepressor effects of small doses of acetylcholine and that stimulation of the central end of the vagus nerve was followed by inhibition of the vasodepressor effect of acetylcholine only in the presence of the hypophysis. Thus, if pituitrin was liberated during a burn, small doses of acetylcholine should have no effect on the blood pressure. In the four experiments performed, normal vasodepressor responses to acetylcholine were seen before, but none occurred after a severe burn. In 2 dogs, in which reflex liberation of pituitary pressor hormone had been precluded by hypophysectomy, no change in the acethylcholine response after a severe burn was observed. In both animals a drop of blood pressure followed the burn.

Following burns in normal dogs the acetylcholine response disappeared and, in severely burned animals, did not return to the control level. In three experiments a normal response to acetylcholine was present after a mild burn although a rise in blood pressure had occurred.

Since the above experiments pointed to the hypophysis as one important cause of the rise of blood pressure following burns, the next logical step in our experiments seemed to be the transfusion of blood from burned animals into assay animals and the testing of the latter for the presence of pituitary pressor hormone with injections of acetylcholine, as described above.

The transfusion of large amounts of blood from burned into normal assay animals suppressed the vasodepressor effects of acetylcholine. Figure 5 represents one typical experiment. Control injections of graded small amounts of acetylcholine produced typical, sharp, transient depressions of blood pressure. After this response had been found to be constant, 250 cc. of fresh heparinized blood from a burned dog was injected intravenously. The acetylcholine response diminished gradually and, 1½ hours after the injection, it was completely absent. This rather slow disappearance of the depressor effects of acetylcholine was entirely different from the effect of pituitrin or pitressin on the typical acetylcholine response (16). In the latter case, inhibition of the acetylcholine response was present shortly after the injection of pituitrin, and it gradually returned to the control response.

The above experiments made us feel that although the pituitary pressor hor-

mone might play a rôle in producing a rise of blood pressure following burns, it could not explain the entire phenomenon. The next question which presented
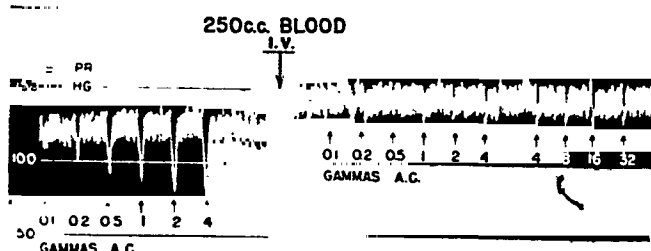
Fig. 5   Fig. 6

Fig. 5. Normal assay dog. Nembutal. Response to A.C. following the injection of blood from a burned dog. At the left side of the record, graded small amounts of acetylcholine iodide produced typical effects. The right part of the record, taken 90 minutes after injection of blood from a burned dog, shows that the effects of acetylcholine were absent.

Fig. 6. Eserinized cat, 3 kgm. Dial. Response of blood pressure to injection of blood from a burned dog.
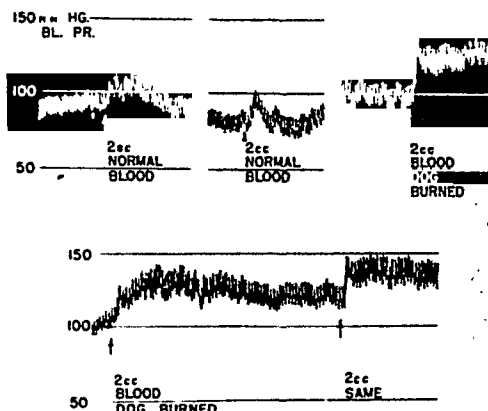
TABLE 2

Injection of blood from burned animals

EXPT. NO.	BURNED-ANIMAL	ASSAY ANIMAL		DRUGS
		Species	Blood press. effect	
			mm. Hg	
PR 1	Dog	Cat	+45	Eserine
PR 2	Dog	Cat	+25	Eserine
PR 4	Dog	Dog	+15	
PR 5	Dog	Dog	+15	
PR 10	Cat	Cat	+20	
PR 12	Dog	Dog	+35	A.C. const. inj.*
PR 13	Dog	Dog	+15	
PR 20	Dog	Dog	+65†	
PR 23	Dog	Dog	+15	A.C. const. inj.
PR 3	Cat	Dog	0	
PR 9	Dog	Dog	0	
PR 10	Cat	Cat	0	
PR 13	Dog	Dog	0	
PR 24	Dog	Dog	0	

* Acetylcholine iodide, constant intravenous injection.
† Large infusion.

itself was whether blood from a burned animal would affect the blood pressure of a normal assay animal.

The upper part of table 2 presents a summary of the experiments dealing with the effect of injections of blood from burned animals into assay animals. In 9

out of 14 experiments a distinct rise in blood pressure from +15 to +65 mm. Hg resulted. The pressor responses were accentuated when the assay animals had been made hypotensive (about 100 mm. Hg mean pressure) with eserine or acetylcholine. Figure 6 represents a typical experiment in which a pressor response was obtained with small quantities of blood (2 cc.); normal blood from the test animal before the burn had only a slight and transitory effect, while the injection of blood drawn after the burn produced a considerable pressor effect, a rise of 25 mm. of blood pressure.

Following burns in dogs, cats and rabbits, an outstanding observation was the immediate appearance of free hemoglobin in the plasma (the question of muscle hemoglobin should be investigated), the amount depending on the severity of the burn. Therefore we had to consider whether normal partly hemolyzed blood per se would affect the blood pressure of an assay animal. The results showed that slightly hemolyzed normal blood, injected intravenously into a normal dog, caused a pressor response. These last findings seem to be important, because one of our most consistent findings has been the presence of hemoglobin in the plasma of burned animals.

Hemolysis in vivo is known to be able to affect kidney secretion. During and following burns, kidney secretion was depressed in all animals, the degree of the depression depending on the extent of the burn. The kidneys of these animals were of chocolate brown colour and were found to be engorged with hemoglobin. It was conceivable, therefore, that an acute hypoxia of the kidneys might follow a burn, and that consequently large amounts of renin would be produced, which would raise the blood pressure. Therefore, acutely nephrectomized animals were subjected to scalding. The typical vasopressor response of burns was found in these experiments.

DISCUSSION AND CONCLUSIONS. We have shown, in confirmation of earlier work, that a reflex mechanism is present in burns, which maintains a more or less normal or even an elevated blood pressure for a considerable length of time. This relatively normal or elevated blood pressure probably explains the rarity of "primary" or "initial" shock (immediate low blood pressure, 10) in humans and animals suffering from severe burns. Wilson (10) in reporting a series of cases of very severe burns stated: "initial shock was infrequent, usually of moderate severity and transient. Profound initial shock was associated with unusual circumstances." However, this relatively normal or even elevated blood pressure gives no indication of the true condition of the animal, for the other experimental findings reveal hemoconcentration, acidosis and increased circulating time. Therefore, if the blood pressure had been the only criterion considered the animal's condition would have been called excellent. The blood pressure can be within normal limits until a few minutes before death. Hematocrit, circulating time and plasma CO_2 values are more reliable indices of damage suffered after thermal trauma. Kabat and Hedin (4, 5) in subacute experiments found slightly longer survival times in spinal animals subjected to a burn than in the case of normal animals. They apparently did not employ a standard degree of thermal trauma; the wide range of the survival times would seem to necessitate a larger series of experiments than employed by them.

We feel that the vasopressor effect of burns had a beneficial effect in the case of our acute experiments; survival times were longer than in comparable experiments with mechanical traumatization, and circulating times were shorter following burns than following mechanical traumatization (13). We feel justified to conclude therefore that, at least in the early stages of burn shock, the maintenance of a more or less normal or of an elevated blood pressure may be beneficial to the organism.

The spleen was excluded as an important factor in the vasopressor effect of burns. This was surprising, because in the dog this organ may contain and expel large amounts of blood.

In an analysis of the reflex mechanisms of the rise of blood pressure in burns, the adrenal medulla and the splanchnic nerves were found not to play an important rôle. Bilateral adrenalectomy modified the curve of the blood pressure rise following a burn, somewhat, but did not abolish it.

The absence, after hypophysectomy, of the typical pressor response following burns does not prove that the hypophysis plays a rôle in the maintenance or rise of blood pressure following a burn, because the organism has been deprived of the tonus hormone of the capillaries; in other words, the reactions of the test animal have been altered. The change in the biologic test with acetylcholine for pituitary pressor hormone was startling, but in no way typical for pituitary pressor hormone. Yet, we cannot dismiss the idea that the considerable degree of hemolysis present in every severe burn could not modify the vasomotor response to acetylcholine to a slowly developing and prolonged reaction.

Our results with normal blood hemolyzed in vitro and with hemolyzed blood from burned animals indicate that, besides the nervous or reflex factor, a physical factor may play a rôle in the pressor phenomenon of burns. In burned animals, kidney secretion slowed down immediately after severe burns and often stopped completely. The kidneys were found engorged with hemoglobin. It was, therefore, conceivable that due to an early hypoxia of the kidneys, renin may enter the circulation. The rôle of renin as a major factor in the vasopressor effect of burns was excluded, however. After having excluded the rôle of the adrenals, the splanchnic nerves, the spleen, and the kidneys as important factors in the vasopressor effects of burns, the following factors remained: hemolyzed blood, pain impulses from the burned area, and the posterior pituitary gland. While the evidence for the first two factors is good, the rôle of the pituitary pressor hormone is not proven, but cannot be ruled out.

SUMMARY

1. During and following burns a rise of blood pressure occurred, which in many experiments remained elevated for a considerable period of time following the burn.

2. This rise in blood pressure may give misleading information concerning the condition of the animal.

3. Scalding of a denervated foot caused a slight drop in blood pressure; scalding of the normal foot resulted in a marked rise in blood pressure.

4. Splenectomy did not abolish the pressor effect of burns.

5. Adrenalectomy, or splanchnectomy, did not abolish the pressor effect of a burn.

6. Hypophysectomized animals exhibited a marked fall in blood pressure when burned, the opposite of the response of normal animals.

7. The acetylcholine vasodepressor response was inhibited in severe burns.

8. A pressor substance could be demonstrated in blood from burned animals, but part of its effect seemed to be due to hemolysis.

9. Renin was excluded as a major factor in the vasopressor effect of burns.

10. The rise of blood pressure following burns seems to be due to a combination of several factors, nervous, hormonal and hemolyzed blood.

REFERENCES

(1) NECHELES, H. AND W. H. OLSON.   Surgery 11: 751, 1942.
(2) NECHELES, H. AND W. H. OLSON.   This Journal 136: 32, 1942.
(3) OLSON, W. H. AND H. NECHELES.   Federation Proc., March, 1942.
(4) KABAT, H. AND R. F. HEDIN.   Proc. Soc. Exper. Biol. and Med. 49: 114, 1942.
(5) KABAT, H. AND R. F. HEDIN.   Surgery 11: 766, 1942.
(6) CRILE, G. W.   An experimental and clinical research into certain problems relating to surgical operation.   J. B. Lippincott Co., 1901, p. 97.
(7) JOHNSON, G. W. AND A. BLALOCK.   Arch. Surg. 23: 855, 1931.
(8) HARKINS, H. N.   Arch. Surg. 31: 71, 1935.
(9) HARKINS, H. N.   The treatment of burns.   Charles C. Thomas, 1942, p. 82.
(10) WILSON, W. C., A. R. MacGREGOR AND C. P. STEWART.   Brit. J. Surg. 25: 826, 1938.
(11) LAMBRET, O. AND J. DRIESSENS.   Rev. de Chir. 75: 319, 1937.
(12) HARTMAN, F. H., W. J. ROSE AND E. P. SMITH.   This Journal 78: 47, 1926.
(13) OLSON, W. H., H. GUTMANN, S. O. LEVINSON AND H. NECHELES.   War Medicine 1: 830, 1941.
(14) BARBOUR, H. G. AND W. F. HAMILTON.   J. Biol. Chem. 69: 625, 1926.
(15) CHANG, H. C., K. F. CHIA, C. H. HSU AND R. K. S. LIM.   Chin. J. Physiol. 12: 309, 1937.
(16) NECHELES, H. AND F. NEUWELT.   This Journal 124: 142, 1938.
(17) GUTMANN, H., W. H. OLSON, S. O. LEVINSON AND H. NECHELES.   This Journal 137: 355, 1942.
(18) O'SHAUGHNESSY, L. AND D. SLOME.   Brit. J. Surg. 22: 589, 1935.

CARDIOVASCULAR ADJUSTMENTS OF MAN IN REST AND WORK DURING EXPOSURE TO DRY HEAT[1]

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It is well recognized that men accustomed to high temperatures can perform fixed physical tasks in the heat with better regulation of body temperature than unacclimatized men. For example, Dill (1-A) demonstrated that a stay of three weeks in the desert produces a marked improvement in the body temperature response to work in the heat. However, there is little evidence as to the rate of acclimatization to the heat or as to the detailed mechanisms involved. All indications are that cardiovascular adjustments are predominant. The cardiovascular studies of Scott et al. (2) were limited to mild heat without physical exertion.

The questions which we have emphasized are: 1. How fast do men acclimatize to heat during the first few days of exposure? 2. How great is the variation between individuals? 3. Can we predict which men will respond best to heat? 4. What is the sequence of the adjustments involved in establishing acclimatization to heat?

These questions have been studied in normal men under rigidly standardized and controlled conditions. This is a report on the cardiovascular findings and related subjects. The principal data comprise some 7,000 observations made on 43 subjects in the course of 202 subject days. Occasional reference is made to results on 23 additional subjects studied during 147 subject days.

Subjects. The subjects used in these experiments were volunteer soldiers from the 710th M. P. Battalion and hired men students of the University of Minnesota. All subjects were free from major physical defects which might affect cardiovascular functions. Ages varied from 18 to 46, most of the men being in the 20- to 30-year age group.

General procedure. Experiments of 3 different durations were used. In the initial series 6 men were studied in an 8-day exposure to the heat. These experiments, together with other evidence, indicated that the major cardiovascular adjustments are completed in about 4 days and that the first 2 days of exposure are critical. Accordingly, a second series was carried out in which 12 men were studied during $3\frac{1}{2}$ days exposure (series II) and 25 men were studied during 2 days' exposure (series III). In all cases the men were also studied under temperate conditions (control) for 2 days immediately prior to the hot periods.

During the hot periods the subjects ate, slept and worked continuously in the controlled condition suite.

A standard fixed regime of diet, observations, physical work, special tests and rest was followed. Standard clothing consisted of cotton shorts, socks and shoes for work and, for evening and rest periods, standard U. S. Army one-piece Armored Forces battle dress. Physical work consisted of marching, in groups of 2 to 4 at a time, on a large motor-driven treadmill. The rate was constant at 3.25 miles per hour at an angle of climb of 7.5 per cent. This work demands oxygen consumption at about 7 times the basal rate. The work regime alternated work and rest in periods of 10 minutes each.

TABLE 1

Protocol for daily routine

TIME	TEMPERATURE	ACTIVITY	OBSERVATIONS
	°F.		
7:30– 8:00 a.m.	85	Basal	Rectal temperature Pulse, weight Crampton test
8:00– 8:30 a.m.	95	Breakfast	
8:30– 9:15 a.m.	105	Rest	Cold pressor test
9:15–12:00 a.m.	110–120	8 work-rest periods, 20 min. each	Work pulse rate Rectal temperature Rate of sweating
12:00–12:30 p.m.	110*	Lunch	
1:30– 4:15 p.m.	120	8 work-rest periods, 20 min. each	Work pulse rate Rectal temperature Rate of sweating
4:35– 5:10 p.m.	120	Rest	Crampton test Cold pressor test
5:10– 6:00 p.m.	110	Rest	
6:00 p.m.	90	Supper	
6:00– 7:30 a.m.	85		

* A 10°F. difference between dining room and treadmill room.

Particular attention was paid to pulse rates in rest and work, rectal temperature, rate of sweating, cardiovascular postural adjustment tests and cold pressor tests. An outline protocol of procedure for a typical day is given in table 1.

Environmental Conditions. The air temperature in the hot periods was 120° F. dry bulb and 85° wet bulb during the day and 85° dry bulb and 65° wet bulb at night. The "effective" temperatures (Houghton and Yaglou, 3) were 92° and 74°, respectively. The control period temperature was the same as at night during the hot periods. The transition from night to day conditions required 2½ hours in the morning; 110°F. was reached within the first hour. The evening transition was completed more rapidly. Air movement was constant at all times. Radiation exchanges were not completely controlled; the wall temperature slowly followed the air temperature, reaching 118°F. in the late afternoons.

METHODS. *Pulse rates* were counted with the aid of a stethoscope in rest and at the end of the work periods on the treadmill. The latter count was begun within one second of cessation of work and continued for 15 seconds. The a.m. and p.m. "work pulse rates" referred to subsequently in this report are the averages of 8 measurements for each man.

Body temperature was measured rectally with a clinical thermometer. Rectal temperatures were measured during the first 90 seconds of recovery after the second, fourth, sixth and eighth work periods of each half day.

Postural circulatory adjustment was studied by means of the "blood ptosis" test of Crampton (4). The subject reclined horizontally on a tilt table until 2 pulse counts agreed within one beat per minute; the blood pressure was then measured. The table was then tilted to an angle of 65° and the pulse rate counted until stabilization was reached as before; the blood pressure was measured at this moment. The original Crampton test score does not provide for changes as large as observed in the heat so a modified scoring system was devised which extends the range.

The "cold pressor" test of Hines and Brown (5) was carried out with measurements of blood pressure and pulse rate before, during and after a 60-second immersion of the left hand in ice water.

Rate of sweating was measured by weight loss of the nude subject after careful drying. The balance used was calibrated to $\frac{1}{4}$ ounce. Rate of sweating was measured over the third work and the succeeding recovery period in the morning and again over the sixth period in the afternoon. The 24-hour sweat volume was calculated according to the formula: sweat loss equals water drunk plus water in food plus water of metabolism minus the sum of urine water plus fecal water plus water lost through the lungs. Water drunk, urine volume and weight of food were measured; the other variables were estimated. The final value was corrected for the 24-hour weight change.

Diet and water. A constant diet was provided by a trained dietitian. It contained 3,100 calories, 95 grams of protein and 120 grams of fat. The diet provided 15 ±2 grams of salt and was adequate, according to ordinary standards, in vitamins. On several experiments the salt intake was reduced to 5.8 ± 2 grams. Water was allowed *ad lib* at all times except during observations and actual work on the treadmill.

RESULTS IN WORK. Pulse rates and rectal temperature responses to work during the 8 days of exposure to heat are given in figure 1. Pulse rates for eight work periods for each man for each half day were averaged and the means for six men appear as a single point in the figure. Similarly, the rectal temperature points in the figure each represent the average of 48 determinations on six men for each half-day period. The pulse rates and rectal temperatures showed marked progressive acclimatization; after four days in the heat, the work was done in the heat with a pulse rate and rectal temperature not far different from that for work in the cold (control). There was little or no change from the 5th to the 8th day in the pulse rate or rectal temperature.

The rate of sweating in work (p.m. values) increased 26 per cent over the 8-day period. However one-half the pulse rate and rectal temperature changes were

completed on the 3rd day with only a 4 per cent increase in the rate of sweating, and one-half of the total increase in the rate of sweating during work took place after all adjustments in temperature regulation as measured by rectal temperatures had taken place.

All these changes took place without marked dehydration. Mean weight changes in grams during the day are given in table 2 along with rates of sweating during work and total 24-hour sweat volumes.

RESULTS IN REST. Results in rest are given for series II since the most striking changes occur during the first 3½ days. Values for pulse rate and blood pressure
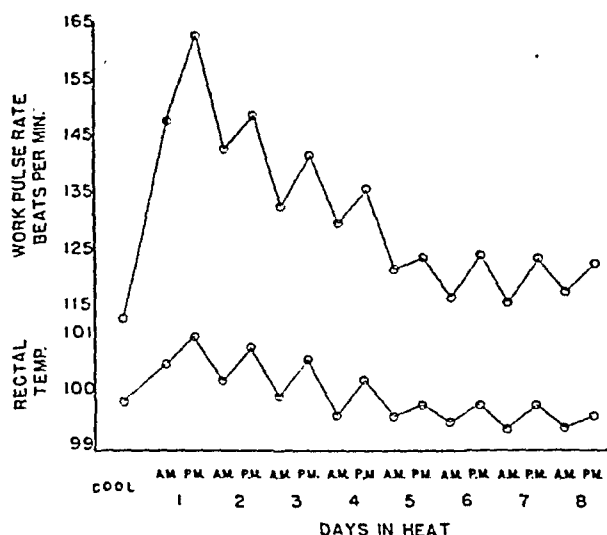
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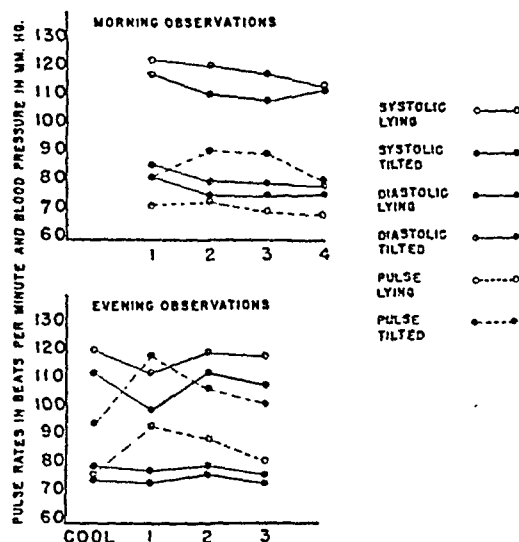
Fig. 2

Fig. 1. The mean work pulse rates and rectal temperatures of six men exposed to eight days of heat.

Fig. 2. The pulse rate and blood pressures of 12 men (series II) during a 3½ day exposure to heat. The upper chart represents basal observations in the morning (85°F.); the lower, observations 20 minutes after work. Note that morning observations on first day of heat serve as a control.

before and after elevation of the tilt table are given in figure 2. Values for 12 men were averaged for morning tests and for afternoon tests.

Marked changes were produced by the heat. In general these alterations tend to return to normal values by the second or third day of exposure to the heat, but the morning systolic blood pressure in the horizontal position seems to be an exception.

Indices of cardiovascular efficiency were calculated by the modified Crampton method (see "Methods"). The average results are listed in table 3; the statistical significance is analyzed in table 4.

Cold pressor test. Information on the state of the autonomic nervous system before, during and after exposure to heat might be expected to be of value, since cardiovascular adjustments to heat depend to a great extent on the proper distribution of the circulating blood. The results of cold pressor tests performed on

15 men in series 3 are summarized in figure 3. Each subject was given 4 trial tests before exposure to heat.

Analysis in terms of Fischer's t values showed the only significant difference occurred between the blood pressure before and during stimulation with ice water in the morning experiment after one day in the heat. A few individual differences were noted. In the 15 subjects studied, two could be classified according to Hines

TABLE 2

The rate of sweating in grams per minute during work, the total 24-hour sweat loss, the weight changes between the 2nd period in the morning and the 6th period in the afternoon, mean body weight, and the 24-hour urine volume

	DAYS IN HEAT				
	1	3	5	7	8
Rate of sweating (gms./min.)					
a.m.	13.3	13.8		14.5	14.1
p.m.	13.4	13.9	15.2	16.7	16.9
24-hour sweat volume (liters)	6.628	6.602	6.468	6.997	6.786
Mean change in wgt. from morning to afternoon* (gms.)	+187	+172	−24	0	−12
Mean body wgt., a.m., (lbs.)	152.8	152.3	152.0	154.4	153.2
24-hour urine vol.	750	809	1,124	919	1,083

* To the 6th period in the afternoon.

TABLE 3

Average Crampton scores (modified) for successive days

	CONTROL DAY	1ST DAY HEAT	2ND DAY HEAT	3RD DAY HEAT	4TH DAY HEAT
Morning		71	61	60	72
Afternoon	60	46	61	56	

TABLE 4

Statistical analysis of cardiovascular fitness indices from table 3

COMPARISONS	DEGREES OF FREEDOM	t VALUE OBTAINED	t VALUE AT 1% LEVEL
Control p.m. vs. 1st heat p.m.	11	5.88	3.10
First heat a.m. vs. 2nd heat a.m.	11	3.51	3.10

and Brown (3) in the normal hyper-reactive group before exposure to heat. After one day in the heat, four new subjects appeared in this group and one dropped out.

Individual variation and interpretation. The average results indicate that adaptation to high temperatures is well started by the second day. However this is not true of a significant number of subjects. Table 5 gives the per cent of men studied from the three series who did not show improvement after the first day as judged by work pulse, rectal temperatures and Crampton score.

Nine men failed to show improvement in the work pulse rate on the second day. Seven of these men had no improvement in rectal temperature and only two showed definite improvement in the Crampton score. Thus 13 per cent of the subjects on the second day had definitely not improved by all the criteria used. On the fourth day one man who failed to show any improvement by any of the criteria used had lost 4.5 per cent of his weight and had undoubtedly handicapped himself by failing to drink sufficient water for his needs. Dehydration was also present in the three other men who failed to show improvement in either the Crampton score or the rectal temperature.

Ten men out of 66 studied in this laboratory suffered heat exhaustion requiring interruption in the work schedule. Four of these exhibited clear cut cases of
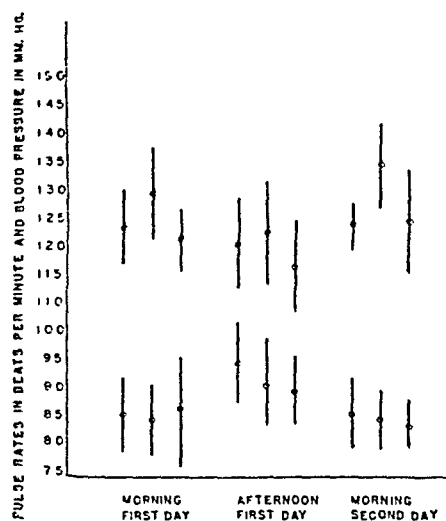
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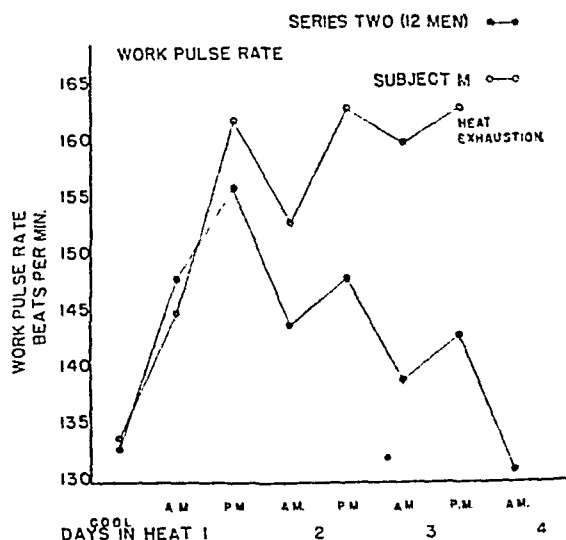
Fig. 4

Fig. 3. Pulse rates, blood pressures and their respective standard deviations of a group of 15 men before, during and after exposure of the hand to ice water for one minute.

Fig. 4. The work pulse rate of subject M who suffered heat exhaustion characterized by hypotension, tachycardia, vertigo and vomiting on the afternoon of the third day in the heat compared to a group of 12 men (series II) who acclimatized in a normal manner.

heat prostration characterized by low resting blood pressure, rapid pulse, nausea, vomiting and vertigo. Three of these cases occurred on the second day in the heat; one on the third day. Figure 4 shows the failure of subject M to have any improvement in his work pulse rate until he finally suffered heat prostration on the afternoon of the third day. The work pulse rates of the other three men who suffered heat exhaustion on the second day were either higher than or equal to the values of the first day. Table 6 gives the average of findings on these four men on the evening of the first day compared with the average findings of the 26 men who were taking part in the experiments which included these cases of heat prostration. The absolute magnitude of the pulse rate does not appear to have much significance but the failure to show an improvement from day to day must be considered as a danger sign.

The men who suffered heat exhaustion and prostration were kept in the high temperature suite. Treatment consisted solely of rest. This proved sufficient to enable all except one man to resume the work schedule within 24 to 30 hours. It is interesting to note that the men who suffered heat prostration or exhaustion did not exhibit excessive thirst at any time.

The Crampton scores for the four men who later suffered heat exhaustion were 5, 5, 25 and 30. Faintings before completion of observations were arbitrarily assigned a value of 5. Two men of the 22 who did not have heat exhaustion had scores of 5 and 30 and were classified by the observers as poor and two had scores of 30 and 25 and were classified as fair.

Evening Crampton scores of 30 or less may be classified as in the danger zone. Individual variations from day to day above this value are difficult to

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TABLE 5

The per cent of subjects studied showing no improvement in pulse rate, rectal temperature and Crampton score after exposures to heat of different duration

AYS IN HEAT COMPARED	NO. OF SUBJECTS STUDIED	PULSE	RECTAL TEMP.	CRAMPTON SCORE
		per cent	per cent	per cent
1st and 2nd day*.	43	21	68	40
1st and 3rd day*..	17	12	24	12
1st and 4th day†..	17	6	18	18
1st and 5th day*..	6	0	0	0

* Basis of comparison p.m. values.

† Basis of comparison a.m. values.

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TABLE 6

Comparison of the average values of work pulse rate, rectal temperature and Crampton scores on the evening of the first day in heat of 4 men who subsequently suffered heat exhaustion and the average values of 26 men exposed to identical conditions

	WORK PULSE RATE	RECTAL TEMP.	CRAMPTON SCORE
		°F.	°F.
Average of 4 men who suffered heat exhaustion	167	101.3	16
Average of 26 men who did not suffer heat exhaustion	166	101.3	43

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interpret. Rank correlation coefficients of Crampton scores on repeat measurements under identical conditions after three preliminary trials were always poor (0.40). Morning Crampton scores did not appear to be a reliable index of condition.

We were unable to select those men who would tolerate heat well by any of the variables measured. No relationship was found in the cold between a, resting pulse rate; b, work pulse rate; c, Crampton score; d, basal rectal temperature; e, work rectal temperature (in the cold) and the ability to perform work in the heat either before or after acclimatization.

DISCUSSION. The strain placed on the circulation by the excessive demands of the skin for an increased blood supply to serve the requirement of heat regulation may be very large. Adolph (6) has estimated that approximately 2.5 liters of blood per minute must be circulated through the skin to dissipate the heat production of walking in the desert. In the unacclimatized man this increased

circulation through the skin produces the early signs of peripheral circulatory collapse, i.e., a rapid work pulse rate (7), a decreased stroke volume and in some cases a decreased minute output (8), and a large increase in pulse rate and drop in blood pressure on assuming the upright position (9, 10). These facts are best explained by the inability of the vasomotor system to make the adjustments necessary to maintain complete filling of the great central veins. The rapid fall of the work pulse rate with continued exposure to heat of our subjects coupled with the marked improvement of the Crampton score indicates that the appropriate vasomotor adjustments are made in large part during the first four days in heat. The results of the cold pressor test, although not striking, suggest that an increased sensitivity of the vasomotor apparatus of the autonomic nervous system plays a rôle in the process of adaptation.

Blood volume changes may be of importance in this process. It is well known that loss of electrolytes and water will lead to a decreased blood volume (14). However, blood volume effects have been minimized by 1, an adequate salt intake, and 2, an adequate water intake. It is felt that the salt intake was adequate since doubling the salt intake did not influence the response or rate of acclimatization to heat (11).

Dill (1-B) has emphasized the rôle that an increased capacity to produce sweat plays in adaptation to high temperatures. The evidence presented here indicates that the 24-hour sweat production is not significantly changed and that the increased rate of sweating during work (see table 2) is a late and relatively minor adjustment. In fact, one-half of the total 26 per cent increase in the rate of sweating appears to serve no useful purpose since it occurred after all the adjustments in temperature regulation (as judged by the rectal temperature) had taken place.

Correlations between rectal temperature and pulse rate first reported by Haldane (12), between pulse rate and environmental temperature (13) and between the rate of sweating and pulse rate (6) will be dependent on the state of acclimatization and must be stated in terms of this variable.

SUMMARY

1. Seven thousand observations on pulse, blood pressure, rectal temperature and rate of sweating in work and rest are reported on 43 subjects (202 subject days) on a constant salt diet before and during exposure to dry heat for 2 to 8 days. Additional observations were made on 23 other subjects for 147 subject days. Observations of pulse and blood pressure before and after elevation on a tilt table were made morning and evening. Modified Crampton scores of cardiovascular fitness were calculated from these figures.

2. Marked deviations from control values in cool conditions were observed in work pulse rates, rectal temperatures and Crampton scores during the first days in heat.

3. Ten cases of heat exhaustion occurred; four of these were clear cut examples showing collapse with hypotension, tachycardia, vertigo and vomiting. Rest without removal from the hot environment sufficed to restore the ability to perform work in these men.

4. A rapid improvement in work pulse rate, rectal temperature and Crampton score took place and was complete in 4 to 5 days. No significant change took place in these variables from the fifth to eighth days.

5. The primary adjustment involved in acclimatization to heat is an improvement in cardiovascular efficiency. A decrease in the accumulation of heat, as measured by the rectal temperature during work, is probably secondary to cardiovascular improvement.

6. The average daily sweat loss is not affected by acclimatization. The rate of sweating during work tends to increase as acclimatization proceeds but a large part (one-half) of this change occurs after the more important adjustments, as indicated by the rectal temperature and pulse rate during work, have taken place.

7. The failure of the work pulse rate to show improvement over the value of the first day is a sign of impending heat exhaustion; similarly, poor cardiovascular postural adjustment in the evening is a danger sign.

8. None of the variables studied in the cold (control) are useful in the prediction of the ability to acclimatize in subsequent exposure in heat.

REFERENCES

(1) DILL, D. B. Life, heat and altitude. Harvard University Press, A, p. 44; B, p. 48, 1938.
(2) SCOTT, J. C., H. C. BAZETT AND G. C. MACKIE. This Journal **129**: 102, 1940.
(3) HOUGHTEN, F. C. AND C. P. YAGLOU. Trans. A. S. H. V. E. **29**: 163, 1923.
(4) CRAMPTON, C. W. Am. J. Med. Sci. **160**: 721, 1920.
(5) HINES, E. A. AND G. E. BROWN. Am. Heart J. **11**: 1, 1936.
(6) ADOLPH, E. F. This Journal **123**: 486, 1938.
(7) DILL, D. B., H. T. EDWARDS, P. S. BAUER AND E. J. LEVENSON. Arbeitsphysiol. **4**: 508, 1931.
(8) ASMUSSEN, E. This Journal **131**: 54, 1940.
(9) KEETON, R. W., F. H. HICK, N. GLICKMAN AND M. MONTGOMERY. Trans. A. S. H. V. E. **46**: 157, 1940.
(10) WEINER, J. S. J. Ind. Hyg. and Toxicol. **20**: 389, 1938.
(11) TAYLOR, H. L., A. F. HENSCHEL AND A. KEYS. Unpublished observations.
(12) NADOL, J. W., S. PEDERSEN AND W. G. MADDOCK. J. Clin. Investigation **20**: 691, 1941.
(13) HALDANE, J. S. J. Hyg. **5**: 494, 1905.
(14) McCONNELL, W. G. AND F. C. HOUGHTEN. Trans. A. S. H. V. E. **29**: 129, 1923.

THE EFFECT OF EXPERIMENTAL HEPATITIS ON THE PLASMA PROTEINS OF THE IMMATURE RAT

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It has been shown that limitation of the diet during the added strain of pregnancy may reduce the protein content of the plasma and that in the presence of experimental hepatitis during pregnancy a marked lowering of the protein content of the plasma occurs. In these experiments it appeared that the drain of protein to the fetus was at the expense of the maternal plasma proteins and was reflected in the lowered level of protein in the plasma when the diet was restricted. Injury of the liver inhibited the formation of plasma albumin and further reduced the level of maternal plasma albumin. Experimental hepatitis and restriction of the diet of the nonpregnant rat are also associated with a lowered plasma protein. The extent of the lowering of the plasma proteins could not be so consistently correlated with the dietary restrictions of the nonpregnant animals as with those of the pregnant animals.

The added requirements for growth of the immature animal are somewhat similar to those of the pregnant animal. In a few preliminary experiments it was found that the administration of carbon tetrachloride to immature rats on restricted intake of food produced a lowering of the plasma proteins similar to that found after exposure to carbon tetrachloride of pregnant rats. The present series of experiments indicate that the protein content of the plasma of immature rats is lowered by the production of experimental hepatitis and that the lowering can be correlated with the amount of restriction of the diet. Attempts were also made to alter the levels of plasma protein in immature animals that had experimental hepatitis by altering the quality of the dietary protein. Unfortunately this phase of the work was interrupted by the war.

PROCEDURE. Individual litters of rats from twenty-one to twenty-six days of age were divided into two groups, one of which was to receive carbon tetrachloride and the other to serve as the control group. The experimental period differed among the various litters, but within each litter both the controls and the treated animals underwent the same regimen except for the carbon tetrachloride. The treated rats were exposed to carbon tetrachloride, 1 cc. vaporized in 10 liters of air, for a ten minute period each day. The average dietary intake of the controls was restricted to coincide with the average intake of the experimental animals each day. At the end of the experimental period the animals were weighed and anesthetized and blood was withdrawn from the heart. Pooled samples of each group were analyzed for total protein and for albumin and globulin by the method of Howe (1). The results of this experiment are given in table 1. All of the figures given are average figures obtained from the two to six rats in each experimental and control group. The food intake is recorded as the average daily intake for each rat.

There are obviously several objections to the procedure outlined in the preceding paragraph. Most of these objections were obviated in similar experiments repeated under paired feeding technic. Two animals of the same litter and the

TABLE 1

Effect of carbon tetrachloride on immature rats

EXPERI-MENTAL PERIOD	BODY WEIGHT		LIVER WEIGHT		FOOD INTAKE		PLASMA TOTAL PROTEIN		PLASMA ALBUMIN		PLASMA GLOBULIN	
	Con-trols	CCL₄	Con-trols	CCL₄	Con-trols	CCL₄	Con-trols	CCL₄	Con-trols	CCL₄	Con-trols	CCL₄
days	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*
31	86.0	77.3	3.77	4.67	6.20	6.16	5.48	3.78	3.85	2.25	1.63	1.53
25	85.6	81.3	4.36	5.46	6.60	7.05	5.40	4.35	4.13	2.54	1.27	1.81
22	66.2	53.7	2.92	4.24	4.42	4.45	5.26	4.13	3.17	2.13	2.09	2.00
21	51.7	54.5	2.57	4.25	4.20	4.83	5.05	3.57	3.07	2.11	1.98	1.46
21	113.0	76.0	5.47	5.38	Ad lib	Ad lib	4.65	3.91	3.45	2.70	1.20	1.21
20	37.7	49.3	2.13	3.14	3.76	3.77	4.01	2.59				
20	57.3	60.0	3.45	4.24	4.05	5.20	4.90	3.84	3.09	1.87	1.81	1.47
18			2.54	5.41	5.40	5.32	5.22	3.60	3.68	2.26	1.54	1.34
18			2.34	3.49	4.25	4.84	4.61	3.88	2.67	2.22	1.94	1.66
18	44.4	45.4	2.25	3.98	4.75	4.70	5.13	4.15	3.64	2.74	1.49	1.41
17	46.5	46.0	2.01	3.59	4.20	4.71	4.84	3.63				
13	59.0	48.5	2.89	3.68	5.00	5.35	4.09	2.35				
12	39.4	32.8	1.81	2.68	3.74	2.92	4.16	3.88				

TABLE 2

Effect of carbon tetrachloride on immature rats: paired feeding technic

EXPERIMENTAL PERIOD	BODY WEIGHT		LIVER WEIGHT		DAILY NITROGEN INTAKE		PLASMA TOTAL PROTEIN	
	Control	CCL₄	Control	CCL₄	Control	CCL₄	Control	CCL₄
days	*grams*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*	*grams per 100 cc.*	*grams per 100 cc.*
19	60	34	2.47	4.03	228	228	4.48	3.41
18	35	47*	1.72	3.40	212	212	5.62	3.19
19	50	46	2.17	3.74	188	188	3.45	2.90
19	47	34	2.25	3.81	183	183	5.40	2.94
18	61	56	2.84	3.76	173	173	3.62	2.88
14	34	48*	1.91	2.65	147	147	4.14	3.44
14	38	53*	2.79	3.26	146	146	4.33	3.62
18	36	54*	1.58	3.52	145	145	3.86	2.66
14	36	45*	1.58	3.28	118	118	4.13	3.60
10	37	27	2.14	1.76	89	89	4.88	2.10

* Ascites present.

same weight were kept in individual cages and allowed free access to water. The food was weighed individually and the dietary intake of each animal was determined by that of its pair mate. The results of this experiment are given in table 2. The nitrogen intake of each animal as given is the daily average for the

period of the experiment. During the first two or three days of the experiment the treated animals ate sparingly. The control animals were likewise restricted. As the experiment progressed, the intake of food was increased.

RESULTS. The livers of the animals receiving carbon tetrachloride were all definitely fatty in appearance in contrast to the normal red color of the livers of the control animals. The livers of the treated animals were also larger than those of the control animals. Hyperbilirubinemia was present in the treated animals and all of them appeared slightly icteric when carefully compared with the control animals. The body weight of the treated animals was less than that of the controls, except for those treated animals in which ascites developed. This may indicate that the utilization of food was impaired by the administration of carbon tetrachloride, as in many cases the intake of food of the treated animals exceeded that of their controls.

The rats which received carbon tetrachloride were unable to attain the levels of plasma protein maintained by the control animals. In comparable experiments the level of plasma protein was lowest in those treated animals receiving the greatest dietary restriction. It seems probable that the levels of plasma protein in the treated animals are the resultant of three components, the dietary intake, the protein requirements of the body and the injury to the liver. The changes produced in the globulin of the plasma were not great. In all cases the reduction of the level of plasma protein was at the expense of the albumin fraction.

Two other series of paired feeding experiments gave results very similar to those given in table 2. In these experiments an attempt was made to determine the effects of altering the quality of the protein in the diet by feeding a third rat for each pair a similar amount of diet except for the replacement of part of the diet with a different protein. In one series the replacement of 20 per cent of the protein with erythrocytes of dogs, a protein which is relatively poor for regeneration of plasma proteins after plasmapheresis (2), did not further lower the plasma protein content of the treated animals. In the other series the replacement of 30 per cent of the protein of the restricted diet with plasma protein did not appreciably increase the plasma protein of the treated animals. These experiments should be repeated with larger amounts of replacement of the diet with proteins thought to be especially favorable for plasma protein regeneration. The failure of these experiments to demonstrate any difference in effect of feeding proteins known to be good or poor for regeneration of plasma protein, however, indicates that the failure of production of plasma protein is due primarily to damage of the liver. The control experiments demonstrate that the quantity and quality of the proteins present in the diet were adequate for maintenance of the levels of plasma protein in the untreated animals.

SUMMARY

Experimental hepatitis produced by exposure of immature rats to carbon tetrachloride and restriction of diet reduces the level of protein of the plasma. The lowered level of plasma protein is almost entirely at the expense of the plasma albumin. There was a voluntary restriction of food intake by the treated animals

but much greater restriction of the diet of untreated animals did not produce comparable lowering of the plasma proteins. In the treated animals. the level of protein in the plasma was roughly proportional to the dietary intake. The level of protein in the plasma, however, probably reflects the functional capacity of the liver more than the dietary intake. Substitution of protein, rated as good or poor for the production of plasma proteins in other experiments, for part of the dietary protein did not alter the levels of plasma protein in the treated animals.

REFERENCES

(1) Howe, P. E. J. Biol. Chem. 49: 109, 1921.
(2) Madden, S. C. and G. H. Whipple. Physiol. Rev. 20: 194, 1940.

THE EFFECT OF EXPERIMENTAL HEPATITIS ON THE PLASMA PROTEINS OF THE PREGNANT RAT

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Factors influencing the regeneration of plasma protein have been successfully studied following plasmapheresis (1). While this method is well suited to larger experimental animals, unfortunately various difficulties make it less practicable for use in studies of the rat. In this regard, the idea presented itself that perhaps the physiologic stress of pregnancy might conceivably be considered as an "internal plasmapheresis." While it is true that "plasmapheresis" must here be construed in a modified sense of the term, since the characteristic replacement of whole blood by cells suspended in saline solution is completely lacking, yet it is appropriate to point out that the end result is of a similar nature. In both procedures there is a strain on the stores of body protein. Moreover, the fact that the strain of pregnancy is necessarily more prolonged than that of true plasmapheresis endows its use with special value. If now it be considered that there exists a dynamic equilibrium between the tissue stores of protein and the proteins of the blood stream, it becomes of interest to speculate concerning the effect of pregnancy on the level of the plasma proteins and, more particularly, whether or not the experimental hepatitis produced by administration of carbon tetrachloride during pregnancy might be reflected in the postpartum level of the plasma proteins.

PROCEDURE. *The effect of carbon tetrachloride during the gestation period.* Feeding experiments have been conducted on paired virgin rats obtained from an inbred stock. The animals were fed a commercial rat food which is adequate for rats. This food served as the basic diet throughout those experiments. After similar preliminary feeding periods designed to equalize the stores of tissue protein, the two animals were inseminated by males of the same stock. The presence of blood and mucus in the vaginal smear within a few days after insemination was indicative of the successful implantation of the ovum. One of the two pregnant animals was then give fifteen minute periods of inhalation of carbon tetrachloride daily during the last two weeks of pregnancy. The food intake of the animal so treated became the limiting factor for the intake permitted to its pair mate.

At the end of the gestation period, the newborn rats were weighed, and this weight subtracted from the maternal pregnant weight indicated the body weight of the adult animal alone. The maternal blood was removed by heart puncture, and the maternal livers were removed and weighed. It is obvious that there is a possibility of several variables in such an experiment. It is felt that these, however, have been controlled in great measure by the following precautions: the choice of virgin animals of similar weight from an inbred stock, the similar pre-

liminary feeding periods, the equal gestation periods, the equalized intakes of food, the production of young approximating the same total weight, and the removal of the maternal blood by the same procedure. Whenever any of these standards were not obtainable within reasonable limits, the experiment was discarded or repeated.

The effect of pregnancy alone. The same precautions were observed as previously. Animals of similar weight from an inbred stock were paired, and one

TABLE 1

The effect of carbon tetrachloride during the gestation period

ANIMAL	POSTPARTUM WEIGHT OF ANIMAL	LIVER WEIGHT	NUMBER OF YOUNG	WEIGHT OF YOUNG	TOTAL INTAKE OF FOOD	TOTAL PROTEIN	ALBUMIN	GLOBULIN	HEMATO-CRIT
	grams	*grams*		*grams*	*grams*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*per cent*
1	202	7.97	8	32	243	5.35	3.42	1.93	34.7
1 (CCl₄)	193	10.80	6	21	243	4.89	2.67	2.22	34.2
2	168	7.02	7	34	165	5.81	4.05	1.76	32.4
2 (CCl₄)	153	9.00	8	40	153	4.87	3.10	1.77	39.2
3	171	6.87	5	35	137	4.74			35.0
3 (CCl₄)	171	7.15	8	41	137	4.35			35.0
4	171	6.87	5	35	137	4.74	3.46	1.28	35.8
4 (CCl₄)	172	7.72	8	42	151	3.73	1.86	1.77	40.7
5	172	6.33	6	21	137	4.92	3.27	1.65	35.5
5 (CCl₄)	193	10.80	6	21	243	4.89	2.67	2.22	34.2
6	169	7.30	9	43	139	4.80	3.09	1.71	34.9
6 (CCl₄)	162	6.99	8	32	128	3.88	1.76	2.12	38.9
7	308	11.85	9	24	375	5.59	3.70	1.89	50.5
7 (CCl₄)	302	14.26	12	46	468	5.35	2.25	3.10	35.1
8	220	10.88	5	27	Ad lib	6.58	3.45	3.13	34.1
8 (CCl₄)	192	10.83	6	18	Ad lib	5.00	3.20	1.80	37.9
9	233	11.14	9	33	218	4.86	2.90	1.96	34.8
9 (CCl₄)	262	13.59	9	33	419	4.07	2.51	1.56	36.1

was chosen for insemination. The food intakes were equilibrated during the experimental period, and following the end of gestation, the body weights, liver weights and blood were obtained in the manner described in the preceding section.

RESULTS. *The effect of carbon tetrachloride during the gestation period.* The usual result of treatment with carbon tetrachloride during pregnancy was to cause lower total protein levels than were present when no carbon tetrachloride had been administered. Hypertrophy of the liver indicated that hepatic damage had occurred in the animals treated with carbon tetrachloride. The decrease of

total plasma protein was due to a specific lowering of albumin and not to any decrease of globulin. In fact, the serum globulin of the animals that had cirrhosis was frequently increased, so that the albumin globulin ratio either approached unity or became less than unity. No consistent effect on the hematocrit was demonstrable. These results are presented in table 1. Perusal of this table indicates that the results can be classed roughly in two groups: 1, those in which approximately the same intake of food was not capable of maintaining the total plasma protein as high when carbon tetrachloride had been administered as when it had not been administered, and 2, those in which, in order to attain nearly the same total plasma protein concentration as its pair mate, the animal that received carbon tetrachloride required much greater quantities of food than

TABLE 2

The effect of pregnancy

ANIMAL	POSTPARTUM WEIGHT OF ANIMAL	LIVER WEIGHT	NUMBER OF YOUNG	WEIGHT OF YOUNG	TOTAL INTAKE OF FOOD	TOTAL PROTEIN	ALBUMIN	GLOBULIN	HEMATO-CRIT
	grams	*grams*		*grams*	*grams*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*per cent*
1 P	171	6.87	5	35	137	4.74	3.46	1.28	35.8
1 NP	178	6.87	0		142	6.23	3.46	2.77	41.6
2 P	169	7.30	9	43	139	4.80	3.09	1.71	34.9
2 NP	154	5.03	0		128	5.26	3.49	1.87	41.7
3 P	172	6.33	6	21	137	4.92	3.27	1.65	35.5
3 NP	175	5.75	0		137	5.52	3.39	2.13	44.7
4 P	233	11.14	9	33	218	4.86	2.90	1.96	34.8
4 NP	222	9.44	0		218	6.47	3.57	2.88	37.0
5 P	202	7.97	8	32	243	5.35	3.42	1.93	34.7
5 NP	200	7.93	0		243	6.27	3.43	2.84	39.5

P = pregnancy, NP = no pregnancy.

its pair mate. Even then, the increase was usually limited to the globulin fraction, and the albumin was not increased by these measures.

The gross appearance of the newborn offspring of the animals treated with carbon tetrachloride was no different from that of the newborn not exposed to this toxin. Histologically, the livers of the young whose mothers had received carbon tetrachloride showed only slight differences, which were not consistent.

The effect of pregnancy alone. The effect of pregnancy alone on the concentration of the plasma proteins was to produce levels lower than were present in nonpregnant animals. In other words, as might be expected, pregnancy is a drain on the maternal store of protein, which is reflected in the levels of the total protein concentration, when the food intake has been limited. Contrary to the findings for the rats treated with carbon tetrachloride, this decrease was not of albumin but of globulin (table 2). The effect of pregnancy was also to decrease the hematrocrit but there was no consistent effect on hypertrophy of the liver.

SUMMARY

Carbon tetrachloride administered to rats during pregnancy causes a lowering of the maternal plasma total protein and the albumin existing immediately post-partum, as compared with pair mates not receiving carbon tetrachloride. The globulin fraction is, on the contrary, frequently increased. Some hypertrophic changes occur in the liver as a result of administration of carbon tetrachloride.

The effect of pregnancy alone, under the conditions of these experiments, is to cause a lowering of the globulin fraction, which is reflected in the total protein level. The concentration of albumin is not markedly affected. There is no demonstrable hypertrophy of the liver.

REFERENCE

(1) MADDEN, S. C. AND G. H. WHIPPLE. Physiol. Rev. 20: 194, 1940.

MOTOR CONTROL OF THE THORACIC DUCT

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Bert and Laffont (1882) showed that electrical stimulation of the mesenteric nerves caused a contraction of the lacteals in a digesting animal, while stimulation of the splanchnic nerves produced a dilatation. Furthermore, stimulation of the vagus resulted in a brief lymphatic dilatation followed by a constriction. Mechanical stimulation of the thoracic duct or of the lacteals elicited a wave of contraction which progressed slowly and which was followed by a wave of dilatation.

Camus and Gley (1894), working on dogs with a perfused thoracic duct, reported that stimulation of the left splanchnic caused a dilatation of the cisterna and a corresponding decrease of its internal pressure. They (1895a) also showed that stimulation of the left thoracic sympathetic chain below the stellate ganglion produced a dilatation of the duct. They concluded that the sympathetic elements in the left thoracic chain and splanchnic nerve include dilator fibers for the duct and the cisterna, respectively. In some of their observations stimulation of these nerves caused a constriction. This discrepancy was explained by the assumption that there are also sympathetic constrictors. Stimulation of the vagus by these observers (1895a) led to a decrease of flow through the thoracic duct in only one instance, which they considered atypical. Pilocarpine, they (1895b) found, caused a constriction of the thoracic duct while atropine had a dilator effect.

Beznák (1937) noted that acetylcholine increases the flow of lymph through the duct. Since the microscopic observation of the intestinal villi revealed a concomitant dilatation of arterioles and capillaries he concluded that the increased thoracic flow was due to an enhanced production of lymph.

The survey of the literature shows that the knowledge of the motor control of the thoracic duct is incomplete. The present observations were made with the purpose of determining the influence of the vagi and of acetylcholine and adrenaline on the motility of the duct and cisterna.

METHOD. Cats were used, anesthetized with dial (Ciba, 0.7 cc. per kgm., intraperitoneally). The animals were kept fasting for 24 hours, and then, two hours before the experiment, were given 60 cc. of olive oil through a gastric tube. In addition, after they were anesthetized, they received an intravenous injection of 10 cc. of dextrose (50 per cent), in order to distend the thoracic duct with lymph. A tracheal cannula was inserted and artificial respiration was administered.

A small segment of the innominate vein, at the region of entrance of the

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thoracic duct, was isolated by ligatures of that vein and all of its branches. A cannula was then inserted into the cisterna, which was approached by a midline abdominal incision and by section of the diaphragm. A mass ligature eliminated all the connections of the cisterna other than that with the duct. The innominate vein was then cannulated and perfusion of the duct was started.

The perfusing fluid was Tyrode solution with 6 per cent arabic gum and at 37°. The pressure (from 30 to 50 cm. of the solution) was regulated by a Mariotte flask and was adjusted to obtain 6 to 9 drops per minute. The drops fell on a Marey tambour connected to another tambour recording on the kymograph.

The thorax was opened widely in order to eliminate changes in the diameter of the cisterna or duct due to respiratory activity. In these conditions a contraction of the lymphatic channels will result first in an increase of the flowing drops due to a decrease of capacity, and then in a decrease of flow, due to increased resistance. Conversely, a dilatation will appear as a decrease followed by an increase of the flow.

RESULTS. In a series of observations acetylcholine or adrenaline was added to the perfusing fluid. The solutions were slowly injected into rubber tubing near the cannula inserted in the cisterna. The addition of acetylcholine (10^{-5} to 10^{-7}) resulted in a decrease of flow through the duct (fig. 1). The latency of the effect was variable but its duration was usually prolonged. It may be inferred that acetylcholine has a constrictor action on the main lymphatic trunk.

Similar injections of adrenaline caused an increase of flow, thus indicating that the hormone has a relaxing action (fig. 2). This relaxing action was also observed when adrenaline was administered in the course of the constrictor response to acetylcholine.

The responses both to acetylcholine and to adrenaline decreased in the course of an experiment; after a few hours they were minimal or negligible. They also decreased considerably if the perfusing fluid became cooler.

In another series of observations the effects of intravenous injections of acetylcholine or adrenaline were recorded. Acetylcholine (0.1 γ) caused a decrease of flow, much as it did when added to the perfusing fluid. This decrease was sometimes preceded by a brief increase which may be attributed to a reduction of capacity (p. 602). Injections of adrenaline (20 γ) resulted in an increase of flow preceded sometimes by a brief decrease. Occasionally adrenaline elicited exclusively a decrease of flow, not followed by an increase. This effect may be explained by assuming an inhibitory action affecting exclusively the cisterna—not the duct.

In a final series of observations the vagi were dissected in the neck and were cut centrally. Electrodes were applied to the peripheral parts. Stimulation with faradic shocks from a Harvard coil with 1.5 volts in the primary circuit, was followed by a decrease of flow. It may be inferred that the vagal nerve impulses, like acetylcholine, exert a constrictor influence.

COMMENT. It is usually assumed that the main factor which regulates the flow of lymph through the thoracic duct is the difference between the positive abdominal and the negative intra-thoracic pressures. There is, however, ana-

tomical evidence that the walls of the cisterna and duct contain smooth muscle elements.   In addition Quénu and Darier (1882) described a nervous plexus of fine nonmyelinated fibers in the walls of the thoracic duct of the dog.   These anatomical data suggest contractile functions under a nervous control.   The present observations confirm this expectation.   Unlike blood vessels, however, which as a rule constrict in response to sympathetic impulses, the thoracic duct and cisterna relax under this influence and contract in response to parasympathetic stimuli.

Three factors determine the flow of lymph through the thoracic duct: a, the "vis a tergo," which depends on the continuous production of lymph; b, the
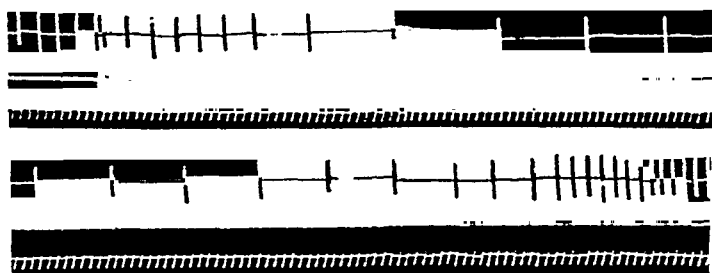
Fig. 1. Decrease of flow through the perfused thoracic duct caused by acetylcholine. Upper record: drops of perfusing fluid.   Middle signal: injection of acetylcholine (1γ) into the fluid.   Lower signal: 5 sec. intervals.   The lower strip follows the upper one immediately.
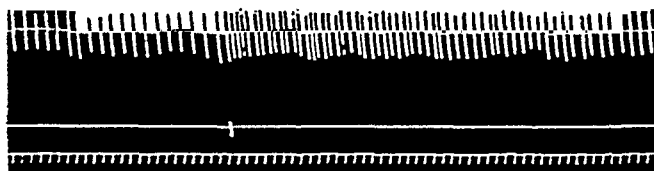
Fig. 2   Fig. 3

Fig. 2. Increase of flow caused by adrenaline.   As in figure 1, but at middle signal adrenaline (10γ) added to the perfusing fluid.

Fig. 3. Decrease of flow caused by vagal stimulation.   As in figure 1, but at the middle signals, first the left and then the right vagi were stimulated peripherally with a coil distance of 12 cm.

pressure gradient between the abdominal and thoracic cavities; and c, the motility of the cisterna and thoracic duct controlled by the autonomic nervous system.

SUMMARY

The cisterna chyli and the venous ending of the thoracic duct were cannulated in cats.   The flow through these channels was measured by recording the number of drops per unit time with a constant head of pressure of a perfusing fluid.

When acetylcholine was added to the perfusing fluid, the flow decreased, indicating a constrictor effect (fig. 1).   Conversely, perfusion with adrenaline resulted in an increased flow, which showed a dilator action (fig. 2).   Intravenous

injection of these substances produced effects similar to those obtained when they were added to the perfusing fluid. Stimulation of the vagus nerve, like acetylcholine, had a constrictor action (fig. 3).

In confirmation of early studies it is inferred that the lymph channels are contractile organs under nervous control. This contractility is one of the factors which governs the flow of lymph.

REFERENCES

BERT, P. AND LAFFONT. Mem. Soc. Biol. 34: 188, 1882.
BEZNÁK, A. B. L. Quart. J. Exper. Physiol. 26: 253, 1937.
CAMUS, L. AND E. GLEY. Arch. de Physiol. 26: 454, 1894.
⠀⠀⠀⠀Ibid. 27: 301, 1895a.
⠀⠀⠀⠀Arch. Internat. Pharmacod. Therap. 1: 487, 1895b.
QUÉNU, E. AND J. DARIER. Mem. Soc. Biol. 39: 529, 1882.

ADRENAL CORTEX AND BLOOD PRESSURE RESPONSE TO CARBON ARC IRRADIATION[1, 2]

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In normal dogs large doses of carbon arc radiation produce a fall in blood pressure (1). This reduction cannot be explained by changes in blood volume (2) or in cardiac output (3), but is believed to be due to a decrease in peripheral resistance brought about by arteriolar dilatation (4). Many observers believe that histamine is set free in the irradiated tissues, absorbed and circulated, producing an extensive arteriolar dilatation which lowers the blood pressure (5). A correlation between an increased content of H-substance in the blood and a reduction in blood pressure following irradiation has been reported (6).

In the absence of the adrenal glands there is a marked impairment of the ability to destroy or inactivate histamine, rendering an adrenalectomized animal many times more susceptible to the effects of histamine than a normal one (7, 8). This resistance to histamine can be restored to normal by the administration of adequate amounts of adrenal cortical substances (9). If the circulatory changes following carbon arc irradiation are due to the production or release of histamine, then such changes should be magnified in an animal possessing no adrenal cortex. To test this hypothesis, dogs were irradiated before and after bilateral adrenalectomy.

PROCEDURE. Adult dogs, ranging in weight from 7 to 14 kilos, were trained to lie quietly on a table for varying periods of time until no excitement was exhibited during blood pressure determinations, withdrawal of blood samples, or operation of the carbon arc lamp. When the period of training was completed, blood pressure, hematocrit, blood sugar, and plasma potassium values were determined until control levels were established.

The auscultatory method of determining blood pressure was employed, the values being recorded from the hind limb. The source of radiation was an Eveready professional model carbon arc lamp, emitting 0.820 gram calorie per square centimeter per minute with a distribution of 5–6 per cent ultraviolet, 29–31 per cent luminous, and 63–66 per cent infra red. The distance of the lamp from the animals and the time of exposure were so adjusted that all experiments were performed with a dosage of 40 gram calories per square centimeter. Ex-

posures were made upon the abdominal region of the dog's body after careful shaving and cleansing of the skin.

The same general procedure was followed in all experiments. The dogs were brought into the laboratory early in the morning, shaved, and placed upon the table. The blood pressure was determined at fifteen minute intervals until two similar consecutive values were obtained. A blood sample was withdrawn, a final blood pressure reading made, and the animal then irradiated. After the irradiation blood pressure readings and blood samples were taken at one or two hour intervals throughout the day. After two or three control experiments, the dogs were doubly adrenalectomized, the operation being performed in two stages under aseptic conditions. The animals were maintained, after adrenalectomy, on desoxycorticosterone acetate (Ciba, Roche-Organon) supplemented at times with adrenal cortical extract (Upjohn, Roche-Organon). Frequent determinations of the blood sugar and potassium as well as blood pressure were the criteria of adequate replacement therapy. After full recovery from the second operation had occurred, the irradiation experiments were repeated exactly as before.

A total of twenty-nine control experiments were performed on eleven dogs. Thirty-eight experiments were repeated on six of these dogs after bilateral adrenalectomy. Twenty-nine experiments were performed on six control animals after the administration of "priming" doses of desoxycorticosterone acetate (D. C. A.) or adrenal cortical extract (A. C. E.). Two dogs which were used in the pretreatment experiments were made hypertensive by application of Goldblatt clamps and again exposed to the carbon arc lamp before and after the administration of D. C. A.

RESULTS. Protocols of typical experiments are shown in table 1. In the control experiments (fig. 1A) the blood pressure began to decline during the first hour following exposure of the animals to the carbon arc. The decline was progressive for six hours following irradiation with recovery gradually taking place after this time. There was a greater proportionate reduction and a more delayed return to normal in the diastolic than in the systolic pressure.

After removal of one adrenal gland the blood pressure response was not appreciably altered from that seen in control experiments (fig. 1C). Following bilateral adrenalectomy, however, a profound increase in the effect of irradiation was produced (fig. 1D). This mainly affected the diastolic pressure; the systolic readings when averaged were almost identical with the values obtained before removal of the adrenal glands. In the instance of the diastolic pressure the average maximal reduction of 23 per cent, occurring six hours after irradiation in the control animals, gave way to an average maximal reduction of 63 per cent occurring five hours after the exposure. In individual experiments the actual reduction of diastolic pressure was often greater than average, and in one instance the animal died thirty-two hours after the irradiation. In this case there was a progressive decrease in diastolic pressure to shock level, an absence of recovery as usually seen, and a failure of the systolic pressure to be maintained. The animal died exhibiting typical symptoms of shock.

It was observed during these experiments that in some instances the adrenal-

TABLE 1

TIME	BLOOD PRESSURE	PULSE RATE PER MIN.	HEMATOCRIT	BLOOD SUGAR	PLASMA POTASSIUM
Experiment 1. 8/19. Control					
	mm. Hg		per cent	mgm. per cent	mgm. per cent
9:00 a.m..................	140/84	68	34	85.8	20.6
Irradiation fifty minutes with carbon arc at one meter					
11:00 a.m...............	126/72	76	34	88.2	21.2
12:00 m.................	118/68	78	35	80.4	20.8
1:30 p.m...............	110/68	76	33	84.8	20.8
2:30 p.m...............	108/60	76	32	80.7	19.8
3:30 p.m...............	108/62	78	32	80.0	21.6
5:30 p.m...............	122/68	72	34	86.7	21.0
24 hrs.................	142/80	70	35	83.4	22.0
Experiment 4. 9/12. After pretreatment					
9:00 a.m.................	146/78	66	34	88.6	21.0
Desoxycorticosterone acetate, 20 mgm., administered intramuscularly. Irradiation fifty minutes with carbon arc at one meter					
11:00 a.m...............	148/82	70	35	85.2	19.8
12:00 m.................	148/76	74	34	86.0	22.4
1:00 p.m...............	152/84	78	34	86.4	22.1
3:00 p.m...............	150/80	76	36	88.0	22.0
5:00 p.m...............	150/84	70	35	88.0	21.7
9:00 p.m...............	148/80	70	34	86.9	19.4
24 hrs.................	148/82	70	34	86.2	20.9
Experiment 8. 10/22. After unilateral adrenalectomy (10/7)					
9:00 a.m.................	142/76	62	33	81.9	21.7
Irradiation fifty minutes with carbon arc at one meter					
11:00 a.m...............	136/62	68	34	80.6	21.4
12:00 m.................	128/56	74	34	84.5	20.6
1:00 p.m...............	116/50	76	33	86.3	22.0
2:00 p.m...............	112/50	76	33	84.0	19.4
3:00 p.m...............	112/50	74	32	80.1	19.6
4:00 p.m...............	110/52	74	30	80.9	20.4
5:00 p.m...............	112/56	70	30	79.6	20.4
8:30 p.m...............	118/64	70	32	82.8	22.8
24 hrs.................	148/72	66	35	86.4	22.1
Experiment 12. 12/27. After double adrenalectomy (12/1). D. C. A. discontinued 48 hours					
9:00 a.m.................	150/82	68	41	78.6	21.3
Irradiation fifty minutes with carbon arc at one meter					
11:00 a.m...............	142/70	72	41	78.2	19.3
12:00 m.................	130/62	84	38	82.9	21.8
1:00 p.m...............	122/42	86	37	84.4	20.7
2:00 p.m...............	116/28	88	36	70.7	22.4
3:00 p.m...............	110/26	96	35	79.3	21.6
4:00 p.m...............	106/18	96	35	76.8	19.5
7:00 p.m...............	106/24	84	36	78.5	19.8
9:00 p.m...............	118/52	76	38	79.6	21.2
24 hrs.................	144/86	70	38	83.2	20.9

ectomized animals failed to show as great a reduction in blood pressure as the controls. This seemed to be correlated with the length of time elapsing between the administration of the maintenance dose of D. C. A. and the beginning of the experiment. Accordingly, it was found that when the animals received D. C. A. up to and including the day of the experiment no great reduction in blood pressure
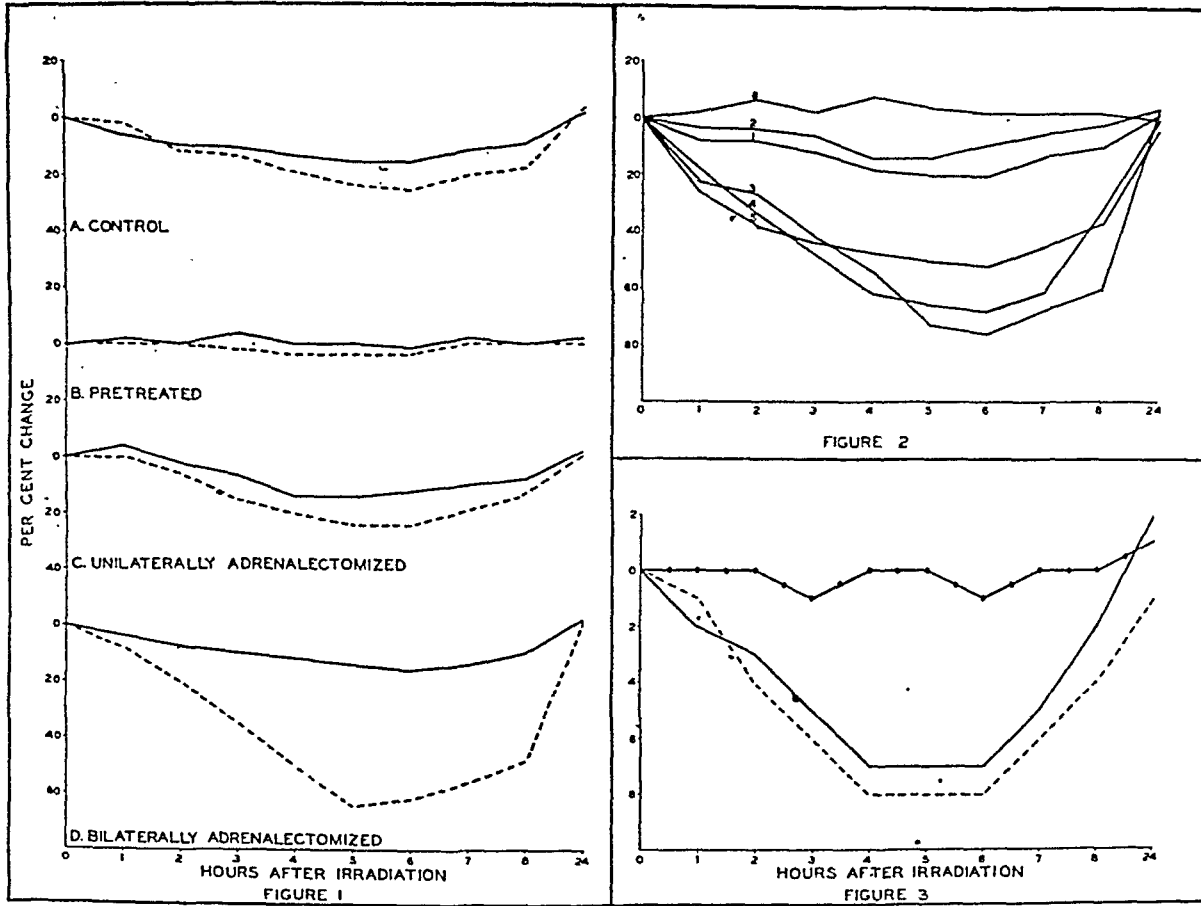
Fig. 1. Curves showing per cent change in blood pressure following carbon arc irradiation. Solid lines indicate systolic, dotted lines diastolic blood pressure.

Fig. 2. Diastolic blood pressure following carbon arc irradiation. *1*, control; *2*, adrenalectomized, D. C. A. not discontinued; *3*, adrenalectomized, D. C. A. discontinued 24 hrs.; *4*, adrenalectomized, D. C. A. discontinued 48 hrs.; *5*, adrenalectomized, D. C. A. discontinued 5 days; *6*, adrenalectomized, D. C. A. discontinued 5 days, with 10 mgm. received on day of experiment.

Fig. 3. Hematocrit changes following carbon arc irradiation. ——— control, ---- bilaterally adrenalectomized, —O—O— pretreated animals.

occurred. The alterations in blood pressure response to irradiation after withholding the maintenance dose of D. C. A. for varying periods of time before the experiment are represented in figure 2. Symptoms of adrenal insufficiency did not appear in most instances until the drug had been discontinued for ten days to two weeks. These observations prompted the experiments on normal animals pre-treated with large doses of D. C. A. or A. C. E. before irradiation. The same

plan as outlined for the control experiments was followed. The irradiations were then repeated after the administration of D. C. A. or A. C. E. (fig. 1B). Such a procedure prevented changes in both systolic and diastolic pressures by the action of the carbon arc. No attempt was made to determine the least amount or optimal dosage of the materials used to produce this effect. It was found that A. C. E. was as efficacious as D. C. A. provided it was administered on the day of the experiment; intravenous administration of the extract twelve hours before experimentation gave no protection to the animal. Intramuscular injection of D. C. A. from one to twelve hours before exposure to the carbon arc was effective in preventing the fall in blood pressure.

In the experience of other workers, carbon arc irradiation has its greatest effect on blood pressures which are initially elevated above the upper limits of normal; for this reason two of the animals used in the experiments just described were made hypertensive. After the establishment of high blood pressures, these animals were irradiated before and after the administration of D. C. A. The responses were essentially the same as those seen at the lower levels of blood pressure.

Figure 3 represents a composite graph of hematocrit changes in all experiments. The dosage of carbon arc irradiation used produced in the normal animal a reduction in red cell concentration, commencing immediately following the exposure, and reaching, after four hours, a maximum which was maintained for the next two hours. Recovery then began to take place and twenty-four hours later there was a normal or slightly higher than normal concentration of red blood corpuscles. After removal of both adrenal glands, the hematocrit determinations followed the same general course from hour to hour as in the control experiments, with no significant intensification of the decrease excepting some delay in recovery to the original values. A higher than initial reading was seldom seen in the adrenalectomized animals twenty-four hours after irradiation, whereas this was usually observed in the normal dog. In normal animals pretreated with adrenal cortical substances there were no significant changes in hematocrit reading following irradiation. This was also true in adrenalectomized animals which were irradiated without a discontinuation of the D. C. A.

Blood sugar and plasma potassium values showed no alterations that were not within the limits of normal physiological fluctuations. The blood sugar in many experiments tended to decrease during the period of maximal blood dilution as indicated by the hematocrit determinations. Fluctuations in plasma potassium followed no general trend and were not regarded as significant.

DISCUSSION. Direct evidence and studies which eliminate other factors have shown that the cause of the fall in blood pressure following carbon arc irradiation is a vasodilatation of the peripheral vessels. This might be produced by reflex nervous mechanisms or by the action of a vasodilator substance. In the light of recent evidence, the protective action of both D. C. A. and A. C. E. in the normal as well as the adrenalectomized animals becomes significant in an analysis of this phenomenon.

From the results obtained it is evident that absence of the adrenal glands

markedly increases the effect of carbon arc radiation on blood pressure. It should be emphasized that the reduced pressure was not accompanied by any change in blood sugar or plasma potassium; the irradiation, therefore, did not produce a typical adrenal insufficiency crisis. These results are in agreement with the concept of Swingle and his co-workers (10, 11) who believe that the secretion of the adrenal cortex is in some way necessary for maintaining the normal ability of the periphery to cope with a vascular strain. Swingle et al. found that large doses of D. C. A. were effective in preventing the shock resulting in adrenalectomized dogs from muscle trauma, intraperitoneal injection of an isotonic solution of glucose, or the injection of large amounts of epinephrine. In contrast, no evidence of a protective action was found against the shock following intestinal stripping in these animals or the circulatory failure following one stage bilateral adrenalectomy. Adrenal cortical extract was effective in preventing shock following any of the procedures. Similar results were obtained by Selye et al. (12) with intact rats, and by Weil and associates (13) with normal rabbits. Swingle and co-workers subsequently showed that the circulation of dogs could be maintained with D. C. A., as well as with A. C. E., following one stage bilateral adrenalectomy provided that the operation was preceded by local blocking of the nerves in the operative field with procaine, by the use of spinal anesthesia, or by section of the spinal cord at the first thoracic vertebra. Spinal section also prevented the circulatory failure following intestinal stripping, and the blood pressure was well maintained by D. C. A. The results seem to indicate the participation of a nervous factor in the circulatory collapse resulting from the procedures. The difference in action of D. C. A. and A. C. E. under these circumstances has been ascribed to the inability of D. C. A. to maintain normal carbohydrate metabolism, resulting in a failure of the peripheral vasculature consequent to the intense reflex stimulation. The frequent occurrence of hypoglycemia under these conditions and the finding that in all types of shock-inducing procedures employed the protective action of D. C. A. was diminished if the adrenalectomized animal had been maintained exclusively on D. C. A. for a long period of time have strengthened this point of view. In the present study, such an explanation for the prophylactic action of adrenal cortical compounds does not seem applicable. D. C. A. was just as effective as A. C. E. in maintaining the circulation in both adrenalectomized and normal dogs. In unprimed animals a hypoglycemia was not associated with the fall in blood pressure.

In view of the complete protection afforded our animals by D. C. A. we do not believe a nervous factor or a disturbance in carbohydrate metabolism is involved. Katz (14) found that the shock produced by massive venous occlusion of the leg in dogs was prevented by pretreatment with D. C. A. In these experiments the rate of fluid loss from the blood stream was diminished by D. C. A., but the ultimate total loss of fluid was not appreciably changed. The protective action of D. C. A. in this instance could not be solely explained by its effect on loss of fluid. In our experiments, D. C. A. and A. C. E. afforded protection against blood pressure changes which appeared in the presence of a blood dilution rather than a hemoconcentration.

It has been suggested (2) that a decrease in the hydrostatic pressure within the blood vessels consequent to a vasodilatation is responsible for the blood dilution. The passage of fluid into the blood stream is insufficient to increase the blood volume enough to restore the blood pressure. This may be associated with a simultaneous increase in the capillary permeability allowing the escape of some of the plasma protein, thereby diminishing to some extent the exchange of fluid. That the adrenal cortical substances did not maintain the blood pressure by allowing a more complete compensation is shown by the close correlation between the hematocrit and blood pressure values. Both begin to decrease in the first hour after irradiation. Both maintain their maximum reductions for about the same time, and the recovery to normal begins almost simultaneously. In the absence of any blood pressure changes there are no significant changes in the hematocrit. Adrenal cortical extract and D. C. A. apparently prevent the initiating factor for such changes, i.e., vasodilatation.

Much evidence has accumulated which indicates that carbon arc irradiation results in the production and release of a vasodilator substance from the irradiated tissues (5, 6). The most probable explanation of the effect of D. C. A. and A. C. E. in these experiments seems to be the protection which such substances are known to exert against the toxic manifestations of histamine. Certainly, whatever substance is produced, it has one property in common with histamine: its action is intensified in the absence of the adrenal cortex and is diminished in the presence of excessive amounts of adrenal cortical substances. It may well be that we are dealing with a complex series of events in which the release, or production, and action of histamine comprise but one important and necessary step in the achievement of the end result.

SUMMARY

1. Irradiation of normal dogs with a carbon arc lamp, using a dosage of 40 gram calories per square centimeter, produced a fall in blood pressure averaging 16 per cent in systolic and 23 per cent in diastolic. After removal of both adrenal glands this reduction averaged 16 per cent for systolic and 63 per cent for diastolic. The fall in pressure could be prevented in normal animals and diminished or prevented in adrenalectomized animals by the administration of D. C. A. or A. C. E.

2. The red blood cell concentration was reduced by an average of 7 per cent following irradiation of the normal dog. After adrenalectomy irradiation reduced the red blood cell concentration by an average of 8 per cent. This reduction, like that of the blood pressure, could be prevented by the administration of adrenal cortical substances.

3. No significant changes in blood sugar or plasma potassium concentration were encountered.

4. It is suggested that the evidence presented further substantiates the view that a humoral mechanism is responsible for the reduction in blood pressure following exposure to the carbon arc lamp.

REFERENCES

(1) LAURENS, H. AND H. S. MAYERSON. This Journal **84**: 524, 1928.

(2) MAYERSON, H. S. AND H. LAURENS. This Journal **86**: 1, 1928.

(3) JOHNSON, J. R., B. E. POLLOCK, H. S. MAYERSON AND H. LAURENS. This Journal **114**: 594, 1936.

(4) LAURENS, H. The physiological action of radiant energy. Chemical Catalog Co., New York, 1933, p. 168.

(5) ELLINGER, F. The biological fundamentals of radiation therapy. Elsevier Publishing Co., New York, 1941, pp. 160, 214.

(6) VON KOLNITZ, H. This Journal **129**: 399, 1940.

(7) BANTING, F. G. AND S. GAIRNS. This Journal **77**: 100, 1926.

(8) ROSE, B. AND S. KARADY. This Journal **126**: 614P, 1939.

(9) ROSE, B. This Journal **127**: 780, 1940.

(10) SWINGLE, W. W., H. W. HAYS, J. W. REMINGTON AND W. D. COLLINGS. This Journal **132**: 249, 1941.

(11) KLEINBERG, W., J. W. REMINGTON, V. A. DRILL AND W. W. SWINGLE. This Journal **137**: 362, 1942.

(12) SELYE, H. Canad. M. A. J. **43**: 1, 1941.

(13) WEIL, F. C., B. ROSE AND J. S. L. BROWNE. Canad. M. A. J. **43**: 8, 1941.

(14) KATZ, L. N., S. T. KILLIAN, R. ASHER AND S. PERLOW. This Journal **137**: 79, 1941.

THE BIOASSAY OF HEPARIN PREPARATIONS

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In the preparation of heparin it is essential to have available an adequate assay method for the determination of the anticoagulant activity of the various fractions obtained. The cat method of assay described by Howell (1) and used by Scott and Charles (2) has been of great value, but is limited in its application because of the variability of the results obtained with individual cats. The various factors that may influence the results have been enumerated by Scott and Charles (2). We have employed the cat method for assaying heparin preparations of varying degrees of activity and found that evaluation of an unknown in terms of the standard was very difficult. This difficulty is due principally to the fact that greater than one hundred per cent increases in heparin concentration are necessary to differentiate between clotted and fluid samples of cat blood.

Because of these difficulties, methods have been described using beef (3) or horse (4) citrated or oxalated plasmas. In a study of citrated plasmas as clotting systems for the measurement of heparin activity, we have found that the best results can be obtained by the use of citrated sheep plasma. The purpose of this paper is to describe a sheep plasma method of relatively high sensitivity for comparing two or more heparin solutions, one of which can be taken as the standard. It has been suggested by Murray and Best (5) that the crystalline barium salt of heparin be assumed to contain 100 units per milligram and used as a standard. The homogeneity and therefore the constancy of anticoagulant activity of this material has been questioned (2, 6). It was subsequently suggested that the barium-free material be taken as a standard (7). Since this confusion exists relative to the choice of a standard we have decided to use the purified sodium salt of heparin put out by the Connaught Laboratories, in solution of 10 mgm. per cc. Several lots of this preparation checked against each other were found to have identical anticoagulant activity. The standard used for the experiments reported here was made from the two lots 86-1 and 87-1.

EXPERIMENTAL. Whole cat blood, recalcified citrated beef plasma, horse plasma and sheep plasma[1] were studied for their suitability as coagulation systems in the assay of heparin.

The cat method of assay. Two sodium salts were assayed by the cat method according to the procedure of Scott and Charles (2). In table 1 are given the results with five cats. Various dilutions of the heparin were made so that when 0.3 cc. was placed in the tube and 0.7 cc. of blood added the assignment of the even whole numbers 100 to 600 could be made. Completely fluid samples are

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[1] We are indebted to Dr. David Klein of the Wilson Laboratories for supplying the sheep plasma.

designated Fl and completely clotted samples Cl. Samples which on inversion slowly ran down the sides of the tube, but were partially clotted, were designated Fl?, and those that were mostly clotted but also could be made to run down the sides with a little fluid present were designated Cl?. The long range of questionable coagulation with the cat blood is apparent from the table. In some instances to go from a fluid to a clot a six-fold dilution of heparin was necessary. The difficulty of evaluating an unknown in terms of the standard by the cat method of assay is therefore obvious. This prompted an investigation of plasma methods for the assay of heparin.

Comparison of horse, beef and sheep plasma clotting systems. For use as a clotting system, the citrated plasmas of horse, beef and sheep have the definite advantage that they can be collected in quantity and stored in the frozen state until ready for use. An ideal plasma for use in the assay of heparin should have the following characteristics: First, the normal clotting time on recalcification

TABLE 1

The assay of sodium salt of heparin by the cat method

UNITS PER MGM.	CAT 6			CAT 7			CAT 8			CAT 9			CAT 10		
	Standard	81A-9	82-9	Standard	81A-9	82-9	Standard	81A-9	82-9	Standard	81A-9	82-9	Standard	81A-9	82-9
100	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl
200	Fl	Fl	Fl	Fl?	Fl?	Fl?	Fl	Fl	Fl	Fl	Fl	Fl	Fl?	Fl	Fl
300	Fl?	Fl?	Fl?	Fl?	Fl?	Fl?	Fl	Fl?	Fl?	Fl	Fl	Fl	Fl?	Fl	Fl
350	Cl?	Cl?	Cl?	Fl?	Fl?	Fl?	Fl?	Cl?	Cl?	Fl	Fl	Fl	Fl?	Fl?	Fl?
400	Cl?	Cl?	Cl?	Fl?	Fl?	Fl?	Cl?	Cl?	Cl?	Cl?	Fl?	Fl?	Fl?	Cl?	Cl?
500	Cl	Cl	Cl	Cl?	Cl?	Cl?	Cl	Cl	Cl	Cl	Cl?	Cl?	Cl?	Cl	Cl
600	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl

should be short,—not greater than three minutes; second, individual samples from the same species should give nearly the same clotting time and, third, there should be a sharp differentiation between clotted and fluid samples at increasing concentrations of heparin. Since normal clotting time did not vary for a period of one year when plasmas were stored at $-10°C.$, all samples were kept at this temperature.

Of 50 individual samples of horse plasma which were available to us, only two were found on recalcification to have clotting times of three minutes or less. The individual variation was very great and therefore the range of heparin concentration would be greatly different for each batch of plasma. Beef and sheep plasmas have desirable coagulation times and show very slight individual variation. Three plasmas were compared with each other with respect to the amount of heparin necessary to cause transition from complete coagulability to complete fluidity. The results of these comparisons are graphically portrayed in figure 1. In each case the optimum amount of calcium chloride for recalcification was found to be 0.1 cc. of a 2 per cent solution per cc. of plasma. After

addition of the desired quantity of heparin and 0.85 per cent saline solution to make a volume of 0.3 cc. in all tubes, 1 cc. of plasma was added, the samples were recalcified and the observations recorded at the end of one hour. At increments of 2 gamma of the standard sodium salt the inhibition of coagulation on recalcification was observed over a range of 4 to 60 gamma. It is apparent that recalcified sheep plasma is the best coagulation system for the quantitative measurement of the anticoagulant activity of unknown heparin preparations. An increase in heparin concentration of 2 gamma produced complete transition from coagulation to fluidity. A sheep plasma method for assaying heparin was therefore set up in which unknown preparations were compared to the standard which was always run in parallel.

The sheep plasma method of assay. Procedure: A series of standard tubes is set up containing 1.2, 1.4, 1.6, 1.8, 2.0 and 2.2 units of standard heparin with
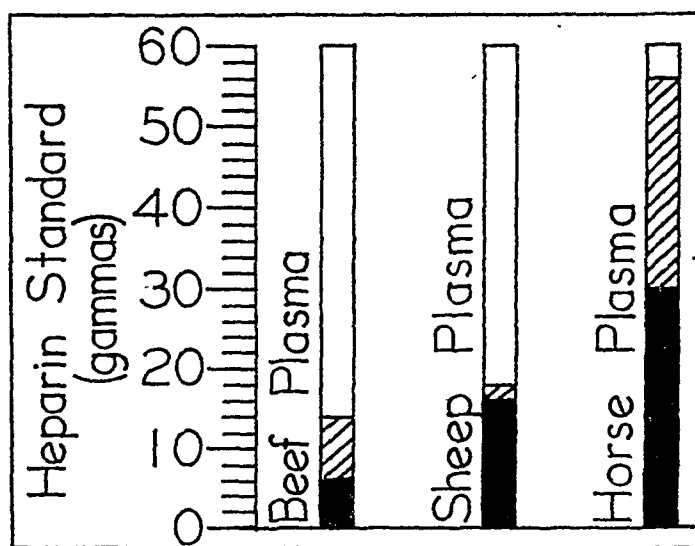
Fig. 1. The inhibition of coagulation of animal plasmas by addition of heparin is graphically portrayed in the figure. Complete fluidity is represented by the clear areas, complete coagulation by the totally black areas and partial coagulation by the cross lines.

the addition of 0.85 per cent sodium chloride solution so that the volume of solution in each tube is 0.3 cc. With most samples of sheep plasma the transition from clot to fluid will occur at the center of this range. It was seldom found necessary to change this range with different sheep plasmas. The addition of more tubes to include concentrations of 1 and 2.4 units may be found desirable. Similarly the unknown heparin solution is set up in a series of tubes covering a range of dosages estimated to be comparable to that of the standard. In case the approximate potency of the preparation to be tested is not known, it may be necessary to run a preliminary assay to determine the range. The volume of the heparin solution in each of the unknown tubes is made up to 0.3 cc. with 0.85 per cent NaCl solution.

One cubic centimeter of citrated sheep plasma is added to each of the tubes. In order that the time factor may be kept constant between standard and un-

known tubes, all series are arranged in ascending order of heparin content with the standard and unknown tubes in parallel rows. Working from left to right, the 2 per cent calcium chloride solution (usually 0.1 cc.) is added from a microburette alternately to standard and unknown tubes. As each solution is calcified, the tube is immediately stoppered with a paraffined cork and mixed by

TABLE 2

Assay of the sodium salt of heparin 185-LBS-1 and of the crude preparation 110-LBS-1

Stock solution of sodium Salt 185-LBS-1: 50 mgm. of powder dissolved in 0.85 per cent NaCl solution q.s. vol. = 50 cc. making 1 mgm./cc.

Stock solution of crude heparin 110-LBS-1: 50 mgm. of powder dissolved in 0.85 per cent NaCl solution, q.s. vol. = 50 cc. making 1 mgm./cc.

Standard heparin solution: 1000 units per cc.

	TUBE NO.	HEPARIN SOLUTION		0.85 PER CENT NaCl	AMOUNT OF HEPARIN	PLASMA	2 PER CENT CaCl	RESULTS 60 MINUTES
		Amount	Dil.					
		cc.		cc.	units	cc.	cc.	
Standard heparin, 1000 units per cc.	1	0.12	1–100	0.18	1.2	1	0.1	Cl
	2	0.14	1–100	0.16	1.4	1	0.1	Cl
	3	0.16	1–100	0.14	1.6	1	0.1	Cl
	4	0.18	1–100	0.12	1.8	1	0.1	Fl
	5	0.20	1–100	0.10	2.0	1	0.1	Fl
	6	0.22	1–100	0.08	2.2	1	0.1	Fl
					mgm.			
Heparin Na salt, 185-LBS-1, 1 mgm./cc.	7	0.12	1–10	0.18	0.012	1	0.1	Cl
	8	0.14	1–10	0.16	0.014	1	0.1	Cl
	9	0.16	1–10	0.14	0.016	1	0.1	Fl
	10	0.18	1–10	0.12	0.018	1	0.1	Fl
	11	0.20	1–10	0.10	0.020	1	0.1	Fl
	12	0.22	1–10	0.08	0.022	1	0.1	Fl
					mgm.			
Crude heparin, 110-LBS-1, 1 mgm./cc.	13	0.08		0.22	0.08	1	0.1	Cl
	14	0.10		0.20	0.10	1	0.1	Cl
	15	0.12		0.18	0.12	1	0.1	Fl?
	16	0.14		0.16	0.14	1	0.1	Fl
	17	0.16		0.14	0.16	1	0.1	Fl
	18	0.18		0.12	0.18	1	0.1	Fl

Calculations: At the end of sixty minutes tube no. 9 of the unknown Na salt series and tube no. 16 of the unknown crude heparin series matched tube no. 4 of the standard series. The Na salt contains therefore, 1.8 units in 0.016 mgm. or 112.5 units per mgm., while the crude heparin contains 1.8 units in 0.14 mgm. or 12.9 units per mgm.

inverting the tube three times. When not more than two unknown series are run in parallel with the standard, this operation requires approximately five minutes.

After recalcification the samples are allowed to stand at room temperature for one hour. At the end of this time they are examined for the extent of coagulation by inverting them, and the results recorded. With sheep plasma, usually

no questionable samples are encountered and never have we had more than one for different batches of sheep plasma. In each series the minimum amount of heparin necessary to prevent clotting is determined and the potency of the unknown solution is expressed in units of standard.

RESULTS. The procedure and calculations are illustrated in table 2 by a specific example in which the sodium salt 185-LBS-1 and the crude preparation 110-LBS-1 were assayed.

In order to compare the values obtained by the sheep plasma method and the cat method, two crude heparins, two barium salts and two purified sodium salts of heparin were tested by both methods. The results obtained using seven cats for each preparation agreed well with those obtained by the use of sheep plasma. The sheep plasma method has been used in controlling the fractionation of heparin from beef lung (8), yielding further evidence for its applicability to preparations showing wide difference in purity.

DISCUSSION. By application of the sheep plasma method here described, the anticoagulant activity of unknown heparin preparations can be quickly determined quantitatively. Since citrated sheep plasma has the property of forming a very firm clot in less than three minutes after recalcification, it is unnecessary to add a thromboplastin solution as MacIntosh (2) found necessary for horse plasma. When unknown heparin preparations are compared against the same standard the results obtained with sheep plasma are quantitatively comparable with those obtained by the cat method, provided several cats are used for each preparation. Thus, the principal advantage of the sheep plasma method lies in its simplicity and greater ease of operation. Comparative assays of the same preparations run independently by different individuals have yielded results which are reproducible within an error of not over 10 per cent.

SUMMARY

A method for assaying heparin has been described, based on the use of recalcified sheep plasma. Studies with horse, beef and sheep plasma indicated that sheep plasma is preferable because it gives a sharp end point with small increments in heparin dosage. The results obtained with the sheep plasma method are comparable to those obtained by the cat method, provided several cats are used. The sheep plasma method is simple, rapid, and yields reproducible results.

REFERENCES

(1) HOWELL, W. H. This Journal 71: 553, 1925.
(2) SCOTT, D. A. AND A. F. CHARLES. J. Biol. Chem. 102: 437, 1933.
(3) REINERT, M. and A. WINTERSTEIN. Arch. Internatl. de Pharmacodyn. 62: 47, 1939.
(4) MacINTOSH, F. C. Biochemical Journal 35: 770, 1941.
(5) MURRAY, W. G. AND C. H. BEST. J. A. M. A. 110: 118, 1938.
(6) JORPES, E. Biochem. J. 36: 203, 1942.
(7) BEST, C. H. British Med. J. 2: 977, 1938.
(8) KUIZENGA, M. H. AND L. B. SPAULDING. J. Biol. Chem. (in press).

TONE AND TENDON REFLEXES AFTER ASPHYXIATION OF THE SPINAL CORD[1]

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The course of recovery of reflex excitability after asphyxiating the spinal cord for various periods of time has been described before (1, 5). In the investigations reported in these papers, reflex activity was examined with only the usual clinical methods. For instance, extensor tone, which is the form of reflex activity returning most frequently after asphyxiation, was judged by the resistance of the leg against flexion. In the present study extensor tone and one of the tendon reflexes (kneejerk) were examined with graphic methods. This makes it possible to follow changes in the excitability of these reflexes more accurately, but makes it impracticable to extend the period of observation beyond 3 to 4 hours after cord asphyxiation. The changes in reflex excitability after that period are known from the aforementioned papers (1, 5).

METHODS. The spinal cord was asphyxiated as described before (1) by forcing Ringer solution in the isolated caudal part of the dural cavity under a pressure higher than the blood pressure. The femur was fixed on a board with screws, and the movements of the lower leg, elicited by contractions of the quadriceps muscle, were recorded by means of trolleys and a lever on a slowly revolving smoked drum. The quadriceps tendon was tapped with an electric hammer (3) at regular intervals. This tapping served the twofold purpose of stimulating the stretch receptors in the quadriceps, thus eliciting the kneejerk and enhancing extensor tone, and also of counteracting friction in the recording system and in the kneejoint which might mask slight changes in tone.

Action potentials, led off from the quadriceps with silver chloride plated silver wires, were amplified and recorded with a Mathews oscillograph.

Extensor tone and kneejerk after cord asphyxiation. Surprisingly soon after asphyxiating the spinal cord for periods up to 65 minutes, a slight and fleeting extensor tone was observed. The first sign of this "initial" tone became visible 4 to 16 minutes after asphyxiation was stopped. In a few minutes it reached a maximum, disappearing entirely 6 to 18 minutes after its beginning (table 1, fig. 1A and B). The initial tone never became high and though it could be found with certainty in the curves, its detection by examining the resistance of the leg against flexion is difficult. This tone has been observed after periods of asphyxiation of the cord ranging from 15 to 65 minutes; after longer asphyxiations (75 to 85 min.) no initial tone developed.

The initial tone was followed in all experiments by a period during which neither tone nor reflexes could be demonstrated (period of areflexia), then reflex

activity started again. In the 65 and 55 minute experiments, a renewed extensor tone was usually the only activity observed. This "secondary" tone began 90 to 140 minutes after the end of asphyxiation (table 1). It reached a maximum

TABLE 1

All the figures, except those in the first column, indicate periods after the end of asphyxiation (minutes). The figures after the plus sign in the column marked "Tone on renewed asphyxiation" have the following meaning. In the 75 and 85 minute experiments the only sign of recovery from asphyxiation was the extensor tone on renewed asphyxiation. The presence of this phenomenon was tested every 20 to 30 minutes. The figures indicate when it was observed for the last time. The "R" in the seventh column indicates that secondary tone remains for the entire period of observation (3 to 4 hrs.).

DURATION OF ASPHYXIATION	INITIAL TONE		KNEEJERK BEGINNING	TONE ON RENEWED ASPHYXIATION	SECONDARY TONE	
	Start	End			Start	End
minutes	minutes	minutes	minutes		minutes	minutes
85				—		
85				—		
85				+ (90')		
75				+ (60')		
75				+ (140')		
75				+ (120')		
65				+	140	170
65	14	30		+ (180')		
65	8	20		+	85	150
55				+	90	R
55	12	30		+	110	200
55	12	28	60	+		
45			130	+	210	R
45	16	22	65	+		
45	8	18	110	+		
35	6	12		—		
35	6	14	50	+		
35			150	+	100	160
25	10	24	90	+	120	R
25	6	18	50		55	130
25			80			
15	12	20	16			
15	6	14	31			
15	4	10	20			

in 15 to 30 minutes and disappeared again completely during the time of observation in three out of four experiments. The secondary tone after these long periods of asphyxiation is much stronger than the initial tone, so strong in fact

that the leg can often only be bent with difficulty. Figure 1A shows the relation between the height of initial and secondary tone. It is known from previous work (1) that after the disappearance of this tone usually no further signs of reflex excitability of the cord are observed during the rest of the animal's life. After the longest periods of asphyxiation (75 and 85 min.), no secondary tone developed.

After the shorter periods of asphyxiation (15 to 45 min., and in one of the 55 min. experiments), the first sign of reflex activity after the period of areflexia was the kneejerk which appeared 16 to 150 minutes after the end of asphyxiation
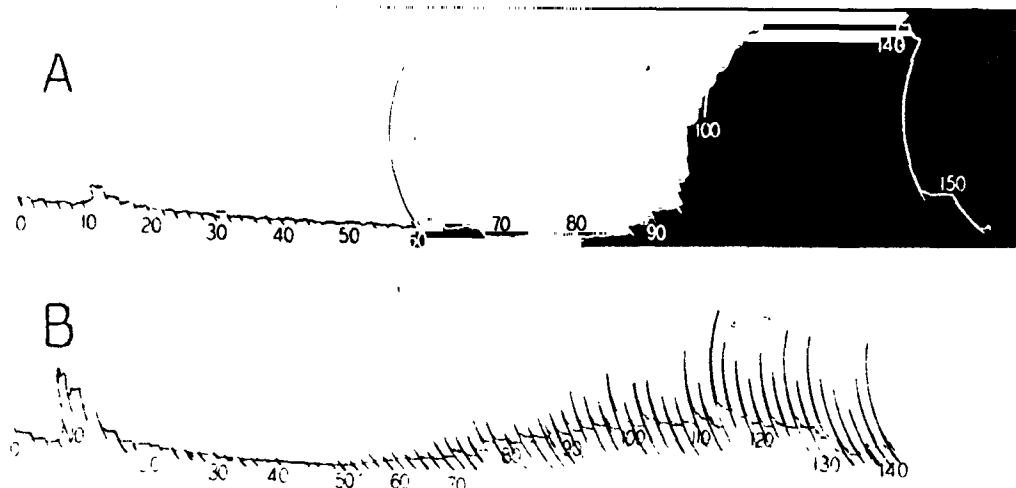
Fig. 1A. Course of quadriceps tone after a 65 minute asphyxiation of the cord. The quadriceps tendon is tapped every 2 minutes. The figures indicate the period after the end of asphyxiation in minutes. After about 8 minutes, initial tone develops which reaches a maximum after 12 to 14 minutes and disappears about 20 minutes after the end of asphyxiation. At 60 minutes the cord is subjected to a renewed asphyxiation of 20 seconds which results in strong extensor tone. After 85 minutes, secondary tone develops which, after reaching a maximum, disappears 150 minutes after the end of asphyxiation. The kymograph has been stopped for about 10 minutes at the maximum of secondary tone.

Fig. 1B. Tone and the kneejerk after 25 minutes of cord asphyxiation. The curve has to be read in the same way as 1A. Six minutes after the end of asphyxiation, initial tone develops which, after reaching a maximum, disappears 18 minutes after asphyxiation. About 50 minutes after the end of asphyxiation, a small kneejerk can be elicited which, after a few minutes, is followed by the development of a slight secondary tone. After about 130 minutes, this tone has disappeared again.

(table 1). After the 35 and 45 minute asphyxiations, this tendon reflex remained small, at least during the 3 to 4 hour period of observation. After the shorter periods of asphyxiation (15 and 25 min.) this reflex increased very quickly (fig. 1B).

In some of the 35 and 45 minute experiments the development of the kneejerk was followed by the appearance of secondary tone. In a previous paper (1) a very pronounced extensor tone has been described after asphyxiations of these durations. However, it usually took longer than a few hours for this strong tone to develop and it is likely that in a number of the present experiments the period

of observation has not been long enough for the secondary tone to appear. It was found previously (1) that this secondary tone was often temporary, like that in the 55 and 65 minute experiments; it not only took longer to develop, but it also remained longer (often 24–72 hrs.). In some experiments this high extensor tone remained for the rest of the animal's life.

In the 15 and 25 minute experiments the reflex excitability usually returned quickly to normalcy after the initial tone and period of areflexia. In the 25 minute experiments a slight and fleeting secondary tone developed before the reflexes became normal (fig. 1B).

The effects of renewed asphyxiation of the cord. During the period of areflexia, extensor tone can be elicited by renewed asphyxiation of the cord. This tone develops and disappears within the course of a minute. If the intradural pressure is not prolonged any more than necessary to demonstrate this phenomenon, it can be repeated each 20 to 30 minutes without apparent effect on the course of reflex excitability after asphyxiation. When the cord was asphyxiated shortly after the disappearance of the initial tone, the extensor tone developed after 20
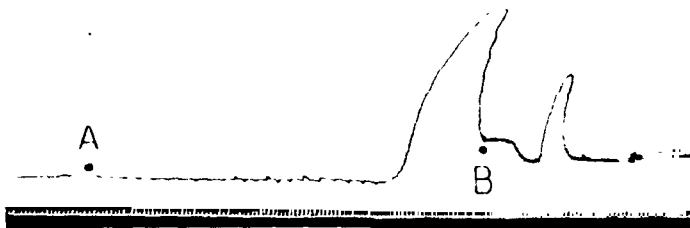
Fig. 2. Extensor tone elicited by renewed asphyxiation during the period of areflexia 70 minutes after the end of a 55 minute cord asphyxiation. Asphyxia is caused by clamping the trachea (at *A*). The clamp is released and artificial respiration is begun after the extensor tone has disappeared (at *B*). The renewed oxygenation causes again a short period of extensor tone. Respiration is visible as small spikes in the curve. Time in seconds.

to 25 seconds and was not very strong. At the end of the period of areflexia, however, asphyxiation caused a very strong extensor tone which sometimes started as soon as 5 seconds after the renewed asphyxiation. The tone reached a maximum in a few seconds and disappeared 15 to 20 seconds after its beginning. Renewed asphyxiation had no effect before the development of the initial tone and after the disappearance of the secondary tone (in the 55 and 65 min. experiments). In all of the 75 minute and in one of the 85 minute experiments extensor tone was elicited by renewed asphyxiation of the cord, though neither initial nor secondary tone develops after these long periods of asphyxiation. The renewed asphyxiation was effective only for a limited period, which corresponded roughly with the period of areflexia in the 65 minute experiments (table 1).

In some of the experiments renewed oxygenation of the cord, by release of the short lasting pressure in the dural cavity, elicited a new period of tone. This is illustrated in the curve of figure 2 in which asphyxiation of the cord was effected by clamping the trachea instead of by forcing Ringer solution into the dural cavity.

The nature of the tone observed. A. *Secondary tone.* It has been found pre-

viously (1) that the extensor tone after asphyxiation, which now can be identified as secondary tone, can be decreased and sometimes even abolished by cutting the dorsal roots relevant to the muscles examined. From this result the conclusion was drawn that this tone is a reflex tone. The following study of the action potentials of the quadriceps muscle during the period of secondary tone supports this conclusion.

With the first signs of secondary tone, small spikes of low frequency became visible in the electrogram. As the tone developed the frequency and size of the action potentials increased until at the maximum of tone large spikes of high frequency were led off from the muscle. When, as in the 55 and 65 minute experiments, the secondary tone disappeared again, the electrical activity diminished and finally disappeared.

With respect to the all-or-none relation, the growth of the spikes, as tone develops, can hardly be caused by a growth of the action potentials of the individual motor units, but is probably caused by an increase in the number of active units. When the tone is slight, only a few motor units will be active and the chances are that none of them is located in the immediate neighborhood of the leading-off electrodes. This accounts for the small action potentials usually found at this stage. As the tone increases, more units become active and the chances that one or more will be situated near the leading off electrodes become greater, resulting in an apparent growth of the action potentials.

The electrical activity during secondary tone can be influenced by changing the length of the quadriceps muscle. During the first development of secondary tone, when hardly any electrical activity was present, spikes could be elicited by flexion of the knee, which stretches the quadriceps (fig. 3A). During a period of moderate tone and electrical activity, the frequency and size of the spikes could be greatly increased by the same stretch stimulus (fig. 3B). When in such experiments the stretch was stopped, a silent period occurred after which the moderate activity was resumed. Also, it was observed that during a period of considerable electrical activity, a relaxation of the muscle by extending the knee resulted in a temporary arrest of all action potentials (fig. 3C).

These reactions of the action potentials on stretch and relaxation definitely characterize the secondary tone as a reflex tone, elicited by impulses from the stretch receptors in the muscle.

Sometimes at the maximum of secondary tone, accompanied by great electrical activity, the influence of stretch and relaxation could not be demonstrated. It is likely that when the reflex activity of the cord is very high, other impulses than those elicited in the stretch receptors can reach the quadriceps motor neurons. Under these circumstances, little effect of changes in the muscle length can be expected.

B. *Initial tone.* Since the interval between the end of asphyxiation and the beginning of the initial tone is too short to sever the dorsal roots, this method cannot be used to establish the nature of this tone. The action potentials of the initial tone, however, can be investigated.

With the first signs of initial tone, spikes appear in the electrogram which

grow in size and frequency with the increase of tone. A maximum is reached in a few minutes which is less pronounced than that during the maximum of secondary tone. Then tone and action potentials decrease and disappear entirely.
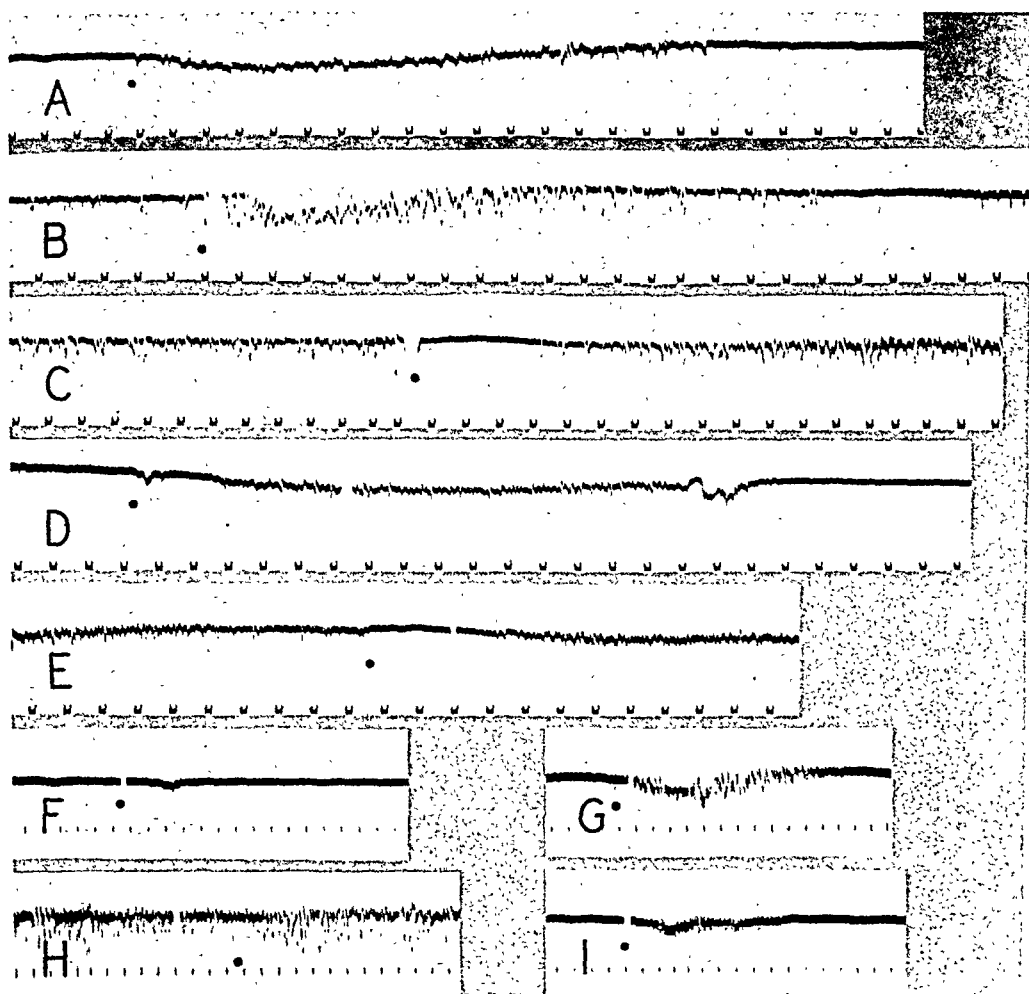
Fig. 3. Effect of bending and stretching the knee on the action potentials of the quadriceps muscle during secondary and initial tone and during the extensor tone caused by renewed cord asphyxiation in the period of areflexia. In each of the curves the beginning of flexion or extension of the knee is indicated by a dot.

A. Seventy minutes after a 50 minute asphyxiation of the cord, when secondary tone begins to develop, flexion of the knee causes considerable electrical activity. B. Twenty minutes later spontaneous activity is greatly enhanced by bending the knee. This increased activity is followed by a silent period. C. Stretching of the knee stops spontaneous electrical activity. This curve is taken a few minutes after B.

D. Eight minutes after a 50 minute cord asphyxiation, when initial tone starts to develop, flexion of the knee causes electrical activity. E. After 10 minutes, continuous electrical activity is present which can be stopped by stretching the knee.

F, G, H, I. During the period of areflexia, 110 minutes after a 50 minute cord asphyxiation, the spinal cord is subjected to renewed asphyxiation. F. Before asphyxiation flexion of the knee has the slightest effect on the electrogram. G, H, I are taken respectively 10, 18 and 30 seconds after the beginning of renewed asphyxiation. G and I show clearly the effect of bending the knee. H shows the great electrical activity at the height of tone. Knee bending has only little effect at that time.

The same influence of stretching (fig. 3D) and relaxation (fig. 3E) as described for the action potentials of the secondary tone has been observed in the electrogram of the initial tone. The initial tone thus can be considered as a reflex tone caused by impulses from the stretch receptors in the muscle.

C. *Tone caused by renewed cord asphyxiation.* The electrogram of the quadriceps during the period between initial and secondary tone did not show any activity. However, at the end of this period stretch of the quadriceps caused in some animals a few small spikes. The strong tone elicited by renewed cord asphyxiation during the period of areflexia was accompanied by the development and subsequent disappearance of large action potentials of high frequency. In some experiments a distinct influence of stretching the quadriceps muscle could be found during the renewed asphyxiation. This is shown in the curves F, G, H, I, of figure 3. Before the renewed asphyxiation, the stretch stimulus had a just visible effect (fig. 3F). This effect had greatly increased 10 seconds after the beginning of the renewed asphyxiation (fig. 3G). The maximum of electrical activity was reached in about 18 seconds after asphyxiation. Stretching the muscle had only little effect at this stage (fig. 3H). After the decline of the action potentials, however, stretch of the muscle again elicited spikes (fig. 3I taken 30 sec. after the beginning of asphyxia). In many experiments the influence of stretch on the action potentials has been less distinct. It can be concluded, however, that the tone elicited by renewed asphyxiation also has the character of a reflex tone.

Tone during renewed cord asphyxiation was not prevented by transection of the dorsal roots supplying the leg. In these experiments the renewed asphyxiation had to be carried out by clamping the trachea since the spinal canal had been opened to sever the roots. It is likely that when the reflex excitability of the cord becomes high enough, the sensory influx from the heterolateral side and from other segment levels is sufficient to produce quadriceps contraction.

DISCUSSION. One of the features of the reflex activity after 55 and 65 minute asphyxiation is that it returns temporarily (1). After the often high secondary tone has disappeared, no reflexes or pronounced tone are observed during the rest of the animal's life. This temporary return of reflex activity has been explained by the assumption that the nervous elements, though being able to function after the asphyxiation, are damaged so severely that they will eventually be totally destroyed. This view is substantiated by metabolic and histologic studies of the cord at various intervals after a 60 minute asphyxiation (2). The first hours after asphyxiation the metabolism of the cord was of almost normal intensity, and the nerve cells showed a normal picture. After a few hours, however, the metabolism decreased and the nerve cells showed changes which, in the next 48 hours, resulted in their total disappearance.

The great height of the secondary tone has been explained by the assumption that the inhibitory structures in the cord are more readily damaged by asphyxiation than the excitatory ones (1). This would result in a disbalance of their respective functions and thus cause an increased reflex excitability of the cord manifest in the high extensor tone. The conception of the high secondary tone as a release phenomenon was based on the observation that it sometimes (es-

pecially after 35 min. asphyxiation) remained for the rest of the animal's life (several weeks).

The following explanations are offered for the course of tone after asphyxiation. The presence of an initial reflex tone 4 to 16 minutes after the end of a 55 to 65 minute asphyxiation shows that synaptic conduction recovers very quickly. It is hardly likely that synaptic conduction, once it has recovered, becomes impossible during the period of areflexia to awaken again during the secondary tone. It is much more likely that synaptic conduction is potentially possible from the beginning of the initial tone until the end of secondary tone, but is depressed during the period of areflexia. This depression may be due to the recovery of inhibitory structures in the cord. The appearance and disappearance of initial tone then is caused by a slight difference in the time of recovery of the excitatory and inhibitory structures in the cord. Thus for a few minutes the excitatory structures are unopposed by inhibition, which results in initial tone. During the period of areflexia the inhibitory structures become so active that a suppression of all reflex activity results. This explanation of the period of areflexia is supported by the observation that reflex activity can be reestablished during this period by renewed asphyxiation of the cord. The renewed asphyxiation would depress the activity of the inhibitory structures before abolishing synaptic conduction, resulting in reflex tone of short duration. When the cord recovers from the renewed asphyxiation, synaptic conduction may recover before the functioning of the inhibitory structures, resulting also in a short period of tone (fig. 2).

The development of secondary tone begins when the inhibitory structures in the cord, which are more sensitive to oxygen lack, are being destroyed as a belated effect of the asphyxiation, thereby leaving the excitatory functions unopposed. This results in the high secondary tone. When a short while later the excitatory structures also are destroyed the secondary tone disappears, not to return for the rest of the animal's life.

A similar explanation can be given for the course of reflex excitability after 45 and 35 minutes' asphyxiation.

It is possible, however, that the depression of the reflex excitability between initial and secondary tone is not due to a recovery of the inhibitory structures in the cord, but is caused by a depression in the excitability of the asphyxiated nervous elements. This possibility is suggested by Lehmann's (4) observation of changes in excitability of excised peripheral nerves after asphyxiation. He found that after a 15 minute asphyxiation the nerve excitability, after an initial recovery, decreases again for a period of about 30 minutes. If it is assumed that similar changes in excitability develop under the conditions of the present experiments in the nervous elements in the cord, this could account for the depression reflex excitability between initial and secondary tone. An explanation of the tone on renewed asphyxiation of the cord during the period of areflexia is, in view of these considerations, not directly obvious.

SUMMARY

1. Five to 15 minutes after the end of a 15 to 65 minute asphyxiation of the spinal cord, a slight extensor tone develops which, after reaching a maximum, disappears within 30 minutes after asphyxiation (initial tone).

2. The initial tone is followed by a period in which neither tone nor other forms of reflex activity are present (period of areflexia). Extensor tone can be elicited during this period by renewed asphyxiation of the cord.

3. In the 65 and 55 minute experiments, a high extensor tone develops after the period of areflexia. This secondary tone is temporary and disappears after a few hours. In the 45 and 35 minute experiments, tendon reflexes develop after the period of areflexia, followed by secondary tone. The secondary tone is usually temporary in these experiments also, but sometimes remains for the rest of the animal's life. In the 25 and 15 minute experiments, the period of areflexia is followed by the development of tendon reflexes and usually the return to normal reflex excitability.

4. It has been possible to prove the reflex nature of initial and secondary tone and of the extensor tone elicited during the period of areflexia by renewed asphyxiation of the cord.

REFERENCES

(1) HARREVELD, A. VAN AND G. MARMONT. J. Neurophysiol. 2: 101, 1939.
(2) HARREVELD, A. VAN AND D. B. TYLER. This Journal 138: 140, 1942.
(3) JOHNSON, C. A. This Journal 82: 75, 1927.
(4) LEHMANN, J. E. This Journal 119: 111, 1937.
(5) TUREEN, L. L. Arch. Neurol. and Psychiat. 35: 789, 1936.

THE PHYSIOLOGICAL RESPONSE TO ENTEROCRININ CONSIDERED QUANTITATIVELY[1]

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Methods for preparing enterocrinin and roughly estimating the amount present in intestinal extracts have been described in a previous paper (Nasset, 1938). The very erratic behaviour of the intestine is quite commonly known among those who have worked with it. The secretory response to intravenous injections of the intestinal hormone, enterocrinin, is no exception; indeed the variability is such as to make very difficult the quantitative determination of enterocrinin potency by any of the types of assay procedures ordinarily used for quick acting hormones and drugs. Since the availability of an accurate and reasonably rapid method of assay is extremely important in any attempt to isolate a physiologically active substance, the intestinal response to enterocrinin was subjected to a rather exhaustive study. The method of assay developed from this study and described below does not differ in fundamental principles from that described earlier, but various refinements in the procedure have greatly increased the accuracy obtainable with a limited number of assays.

METHODS. Anesthetized[3] and dewormed dogs weighing about 20 kgm. were used for all assays. Two adjacent 15 cm. loops of the jejunum were isolated just below the ligament of Treitz and flushed out with warm isotonic saline. Close-wound aluminum wire coils with glass cannulae taped to both ends were inserted as gently as possible and the loops securely tied to the taped ends of the coils. The loops, supported outside of the abdomen, were maintained in good condition with a moist chamber similar to that described by Nasset and Parry (1934).

A thermocouple was inserted into the lumen of one loop through a T-cannula, one end of each loop connected to a drain flask, and the other end connected to reservoir and drop recorder system such as that shown in figure 1. All air was flushed out of the loops by warm saline from reservoir C, the drain tubes closed, reservoir D filled with saline, and A and D connected. The reservoir system was lowered until the dropper E was about 10 cm. below the level of the loops and allowed at least thirty minutes to come to equilibrium. The platinum foil electrodes F were placed in series with a 100-watt lamp and an ordinary kymograph signal magnet in a 110 volt D.C. circuit to function as a very simple but extremely efficient drop recorder capable of recording almost any rate of flow short of a steady stream.

The carotid artery was cannulated and connected to a recording blood pressure manometer. A femoral vein was cannulated and attached to a saline-filled burette to facilitate the injection of hormone preparations.
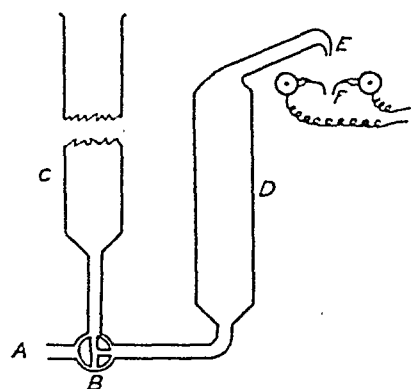
Fig. 1

Fig. 2

Fig. 1. *A*—connection to loop; *B*—3-way stopcock, 4 mm. bore; *C*—200 ml. saline reservoir; *D*—75 ml. reservoir; *E*—dropper, delivers 33 to 34 drops/ml.; *F*—heavy platinum foil electrodes.
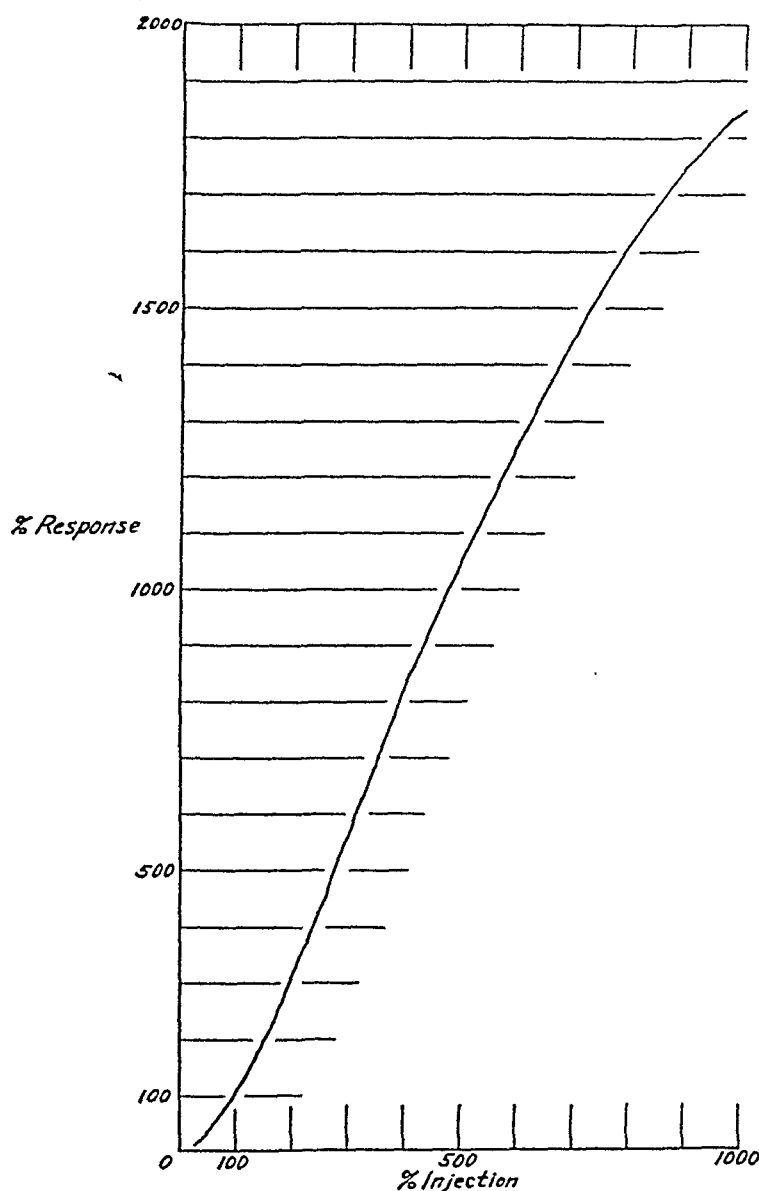
Fig. 2. Curve of response to enterocrinin

If it was desired to estimate the amount of secretin in the preparations to be assayed, the major pancreatic duct was cannulated and attached to a drop recorder before the intestinal loops were isolated.

The temperature of the abdominal chamber was adjusted by means of a rheostat to maintain a loop temperature of about 38°C.

The level of the droppers was adjusted until a low rate of apparent secretion (2 drops/min., or less) was obtained from both loops (the two droppers being always moved together, so that the loops were under equal negative pressures). After allowing the loops about five minutes to adjust themselves to the new pressure, the kymograph was started and this basal, or control, rate recorded for at least five minutes. An injection of a standard enterocrinin preparation was then made and the kymograph recording continued for at least five minutes longer than the period, p, required for the rate of secretion to return to a basal rate in both loops (usually 15 min. was sufficient). An amount of an unknown preparation estimated to be equivalent to the standard injection was then administered. After the secretory rate had again returned to a basal rate and remained there for at least five minutes, a second standard injection was made and the recording continued as before. This group of three injections comprised the basic assay unit. The number of drops in the five-minute period preceding each injection was recorded as a control, C, value, and the number of drops obtained between the time of an injection and the beginning of the next control period was recorded as total, T, value. Each unit assay, then, involved the recording of 7 values for each of the two loops, as follows: 1, control; 2, total for standard; 3, control; 4, total for "unknown"; 5, control; 6, total for standard; 7, control. When a T value referred to a ten minute period, the sum of the previous and the subsequent C values was subtracted from it in order to determine the net response, N, to the injection. When the period was greater than ten minutes the sum of the C values was multiplied by an appropriate factor before subtracting. That is:

$$N = T - (C_1 + C_2)\frac{p}{10}$$

where N is the net response to an injection, T is the total number of drops in the period following the injection, C_1 and C_2 are the previous and subsequent five-minute control values, and p is the period (in minutes) used in determining T. The net response to the unknown preparation was then divided by the average of the net responses to the previous and subsequent standard injections, and the quotient multiplied by 100 to give the response to the unknown as a percentage of the standard responses used, giving each standard a weight in inverse proportion to the interval between it and the unknown injection, as follows:

$$R = \frac{100\, Nu\, (Z_1 + Z_2)}{Ns_1 Z_2 + Ns_2 Z_1}$$

where R is the per cent response of the unknown in terms of the standard, Nu, Ns_1 and Ns_2 are the net responses to the unknown and the previous and subsequent standards, respectively, Z_1 is the time interval between the injection of the previous standard and the injection of the unknown, and Z_2 is the interval between the injection of the unknown and the injection of the subsequent standard.

This method of comparing two preparations is called the "double control, double standard" method, and it, more than any other single factor, is to be

credited with making the highly variable intestinal response yield accurate and reproducible assay results. The method estimates the "expected" control value during the period in which an injection is acting, and the "expected" standard response during the period in which an "unknown" injection is acting, by assuming that the basal rate and the responsiveness to enterocrinin remain constant or increase or decrease at a uniform rate over the periods involved. In a number of tests, the assumption has been found to be sufficiently valid to make the "double control, double standard" method of comparison highly superior to any other method tried.

If the average net response to the standard $\frac{Ns_1 + Ns_2}{2}$ is approximately 10 drops, the enterocrinin curve of response (fig. 2) is entered through the ordinate with the value of R as determined above, and the corresponding per cent injection (I) value read off on the abscissa. This figure is an estimate of the amount of enterocrinin in the dose of unknown expressed as a percentage of that present in the standard dose. When the average net response to the standard differs considerably from 10 drops, the curve of response is entered twice, once at the ordinate equal to ten times the average net response to the standard, and again at the ordinate equal to ten times the net response to the unknown injection ($10\ Nu$). The corresponding abscissa values in this case relate both the standard and the unknown to the point (100, 100) on the curve, and the value of I is found by dividing the abscissa value for the unknown by that for the standard and multiplying by 100. Since a large portion of the central part of the curve closely approximates a straight line this method is used only when the standard response is very large or very small.

The curve of response is an average constructed from data collected during routine enterocrinin assays on more than fifty dogs. All series of graded doses of a single preparation given to the same dog in a short time were assembled, the response in each series nearest to ten drops (0.3 cc.) was taken as 100 per cent response, and the corresponding dose taken as 100 per cent injection. The rest of the values in each series were expressed in terms of this fixed point, and in this manner over 300 points were obtained for the curve, about 100 of which were arbitrarily fixed at the point (100, 100). The extremities of the average curve may differ widely from the curves of response for individual dogs, but the variation becomes much less as the point (100, 100) is approached since all individual curves meet at that point and vary only in slope and linearity. In order to facilitate reproduction, the following empirical equations for the average curve were derived:

(A) $$R = (0.191\ I - 0.9)^{1.59}$$

(B) $$R = 0.1418\ I^{\frac{1}{0.565 + 9.34I \cdot 10^{-5}}}$$

(A) gives a very good fit from the point (35, 15) to the point (200, 315), while (B) gives a fairly good fit over the whole range up to the point (1000, 1840). Obviously, no physiological significance can be attached to such equations.

The per cent potency, P, of the unknown as compared with the standard was calculated by averaging the I values as determined for the two loops, dividing this average by the dose of unknown in milligrams (Du) and multiplying by the dose of standard in milligrams (Ds); i.e.,

$$P = \frac{(I_u + I_1)D_s}{2\,Du}$$

where I_u and I_1 refer to the I values from the upper and lower jejunal loops, respectively.

Either the average I values or P was used to calculate a dose of unknown which should equal the activity of the standard dose. If the responses were still considerably different in the second assay of the unknown, the results of this assay were used to calculate a third dose of unknown, and so on until two values of P checked within ± 10 per cent. Ordinarily, such a check may be obtained with three assays of a previously untested preparation.

When a number of unknown preparations are to be assayed against the same standard, the unknowns are assayed alternately in order to permit the assays to proceed while subsequent doses are being calculated. In this way n assays may be carried out using $n + 1$ doses of the standard and usually at the rate of about two assays per hour. The dose of standard may be varied to keep the response within workable limits without breaking the chain of alternate standard and unknown injections. In this case P is calculated from the previous and subsequent standard responses individually and the values so obtained are then averaged.

Inasmuch as enterocrinin has not as yet been prepared in a pure state and no large stock of a stable standard preparation is available for distribution, it was desired to relate the expression of potency to the absolute value of the physiological response. This was accomplished by setting one preparation aside as a "permanent standard" and testing it occasionally in the animals used for routine assays. After each test the average curve of response was used in estimating the amount of the preparation required to elicit the "threshold response" of 10 drops (0.3 cc.) over the basal rate (calculated from the previous and subsequent control values, as described above). The mean "threshold dose" ($T.D.$) of the "permanent standard", as determined from 42 such tests in 11 dogs, was 52 mgm., with the probable error of the mean ($P.E_M.$) being ± 4 mgm. The preparation showed no evidence of instability during the 12-month period covered by the tests. The stability was further verified by the fact that the coefficient of correlation, r, between the various values of the $T.D.$, and the age of the preparation at the time the values were determined, was only $+0.02$, $P.E_r$ ± 0.10. Any serious decrease in potency should have resulted in a significant, positive value of r. The preparation was stored over $CaCl_2$ at atmospheric pressure. Highly purified preparations were found to be much less stable.

Unknown preparations were assayed against the permanent standard, and their threshold doses determined as follows:

$$T.D._u = \frac{T.D._s \times P}{100}$$

where $T.D.u$ and $T.D.s$ are the threshold doses of the unknown and the standard, respectively. These preparations could then be used as temporary, or assay, standards for assigning $T.D.$ values to other preparations.

The question as to whether the dose should be expressed in terms of the animal's body weight (e.g., as mgm./kgm.) was settled statistically as shown in table 1. The statistics shown involve all the data available on one preparation. The term dose $\cdot$ Wt.$^{-1.0}$ refers to the dose expressed as mgm./kgm. of body weight, while the term dose $\cdot$ Wt.$^{0.0}$ refers to the dose expressed in milligrams, without reference to body weight. The weight enters to varying degrees in the other terms. Inasmuch as the odds are about 14,000 to 1 against a difference such as that between the first and fourth r values occurring by chance ($P = 0.00007$), the expression of the dose without reference to body weight is clearly preferable. The correlation ratios show the same trend as the correlation coefficients, and incidentally prove that the curve of response to enterocrinin is significantly non-linear. Analyses similar to the above showed that the dose of secretin is likewise best expressed without reference to body weight, while the dose

TABLE 1

Correlation of the net response with the dose expressed in various ways

(Number of paired observations, 160; number of dogs, 9; weight range, 13 to 30 kgm.)

X	Y	r_{xy}
Net (drops)	Dose $\cdot$ Wt.$^{-1.0}$	+0.24
Net (drops)	Dose $\cdot$ Wt.$^{-.67}$	+0.34
Net (drops)	Dose $\cdot$ Wt.$^{-.33}$	+0.42
Net (drops)	Dose $\cdot$ Wt.$^{0.0}$	+0.45
Net (drops)	Dose $\cdot$ Wt.$^{+.33}$	+0.38

of the vasodilatins present in intestinal extracts should be related to body weight (e.g., as mgm./kgm.).

DISCUSSION. The highly corrected and controlled method of assay described above was made necessary by the high degree of variability of the intestinal secretory response. The description of the method seems rather involved, but in practice it is really quite workable, requiring little more time and material than a number of less precise assay schemes in use.

The "double control, double standard" procedure has been found quite useful in the assay of secretin and vasodilatins, though in these instances it is not so important as in the case of enterocrinin, for the variations in responsiveness are neither so marked nor so rapid. The method is believed to be worthy of consideration for many procedures where period to period variations in responsiveness are encountered.

Although the statistical analysis showed that it was better not to base the dose of enterocrinin upon the weight of the dog being used, certain theoretical considerations make it appear probable that the response to a given amount of a preparation is not completely unrelated to the body weight, and it is for this reason that a reasonable effort is made to keep the weight near a standard value

of about 20 kgm.   This question is purely academic as far as accurate comparison of standard and unknown preparations is concerned, but it is important in establishing absolute, or permanent standards.

Vasodilatins have relatively little effect upon the assay results.   The secretory response to a powerful vasodilatin injection comes almost immediately as a short spurt of secretion and is followed by a period in which the basal rate is reduced, so that these two periods nearly cancel.   For very accurate assays of strongly vasodilatory preparations, the enterocrinin part of the preparation may be inactivated by treatment with alkali, injected before and after the injection of the active preparation, and the previous and subsequent periods treated as control values.

The parallelism of enzyme and volume responses reported by Nasset and Pierce (1935) has been confirmed using highly potent enterocrinin preparations.   The enzyme response tends to return to a basal level more slowly than the volume response, particularly when large doses are given.   In one series that was analyzed statistically, the coefficient of correlation between the volume and the invertase responses was $+0.84$, between volume and phosphatase $+0.82$, and between invertase and phosphatase $+0.99$.

SUMMARY

A detailed procedure is given for the bioassay of enterocrinin, the hormone which excites the secretory glands of the intestine.   The principal feature of the method, which permits accurate and reproducible results to be obtained quickly from the highly variable intestinal secretory response, is its precise treatment of the time factor in the normal variations in responsiveness.   The general procedure outlined may well be of value in increasing the accuracy and practicability of other types of bioassays.

An average curve of response to enterocrinin is used to assist in equilibrating the responses to the standard and the "unknown" preparations.

A statistical analysis of data on 9 dogs shows a closer dose : response correlation when the dose of enterocrinin is given without reference to the weight of the dog than when it is given on a *per kilo weight* basis.   Secretin, likewise, was found to give more uniform responses in different dogs if the same dose were given, regardless of body weight, while the vasodilatins present in intestinal extracts are best expressed on a *per kilo* basis.

REFERENCES

NASSET, E. S.   This Journal 121: 481, 1938.
NASSET, E. S. AND A. N. PARRY.   This Journal 109: 614, 1934.
NASSET, E. S. AND H. B. PIERCE.   This Journal 113: 568, 1935.

THE FRACTIONATION OF ENTEROCRININ PREPARATIONS[1]

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Methods of preparing enterocrinin and testing for its presence in intestinal extracts have been described in a previous paper (Nasset, 1938). A description of an accurate and rapid method for assaying the hormone is given in the accompanying paper. Since there have been some changes made in the basic fractionation procedure along with the addition of new steps, the improved methods that are now in use will be described in full.

The small intestines of hogs (or dogs or cattle) were removed immediately after death of the animals, turned inside out, washed rapidly in water, and extracted with 87 per cent alcohol containing 3.3 cc. of concentrated HCl per liter (3 l./kgm. of tissue). The extraction was continued for 72 hours, with occasional stirring, at about 5°C. The alcohol solution (fraction 2) was decanted, allowed to settle, and the supernatant concentrated to $\frac{1}{6}$ its volume under reduced pressure at about 40°C. (A "foam-head still" was a great help in this step.) NaCl (300 grams/liter) was dissolved in the crude water solution (fraction 4) while it was still warm, and the mixture set in the cold room overnight. The precipitate was filtered off and removed from the filter while still damp. It was then pressed or pounded between successive layers of filter paper, until no more moisture could be expressed. (This step usually removed the major portion of the vasodilatins.) The precipitate was further dried in vacuo. The dry preparation was shaken with glacial acetic acid (10 cc./gram) for about three hours and then with 5 cc./gram for about one hour. The preparation was precipitated from the acetic acid solution by addition of 4 volumes of an ether mixture (3 parts ethyl, 1 part petroleum), washed twice with ether and once with acetone and dried *in vacuo*. The dry product (fraction 10) was extracted twice with acetic acid (15 cc./gram) and reprecipitated, washed, and dried as above. The dry, white powder (fraction 14) was shaken for several hours with methyl cellosolve (0.07 cc./mgm.), one-half volume of ethyl ether added very slowly with stirring, the mixture covered to prevent evaporation, allowed to stand about an hour at room temperature, and then centrifuged. The precipitate was discarded and to the supernatant was added $2\frac{1}{2}$ volumes of ethyl ether and one volume of petroleum ether (both based on the original volume of methyl cellosolve used). The mixture was allowed to stand several hours in the cold, centrifuged, and the precipitate (fraction 18) washed and dried as above. Fraction 18 was dissolved

in propylene glycol (0.1 cc./mgm.), 8 to 10 volumes of acetone added slowly, with stirring, placed in the cold room overnight, centrifuged, and the precipitate (fraction 20) washed three times with acetone and dried *in vacuo*. The fractionation from acetic acid, methyl cellosolve, and propylene glycol may be repeated, if desired, with little loss of active material. Fraction 20 was dissolved in water (0.02 cc./mgm.), and $1\frac{1}{2}$ vols. of a saturated water solution of picric acid added slowly. The mixture was set in the cold room for 24 to 72 hours and then centrifuged. The precipitate was shaken with two volumes of acetone (this and all further volumes to be based on the original volume of water used with fraction 20), the insoluble residue dried *in vacuo*, and then extracted three times with two-volume portions of hot water (about 90°C.). The combined water extracts were evaporated to dryness *in vacuo*, the residue extracted with two volumes of warm glacial acetic acid (about 60°C.), the insoluble residue discarded, and the solution allowed to cool to about room temperature. The yellow picrate may be reprecipitated at this point by addition of ether, but there is usually little advantage in doing so. HCl gas was bubbled through the acetic acid solution of the picrate until the yellow color disappeared, and eight volumes of ether added. The mixture was set in the cold for several hours, centrifuged, and the precipitate (fraction 30) washed with ether containing HCl gas, with ether alone, and with acetone. It was then dried *in vacuo* and stored over $CaCl_2$ either *in vacuo* or in an atmosphere of nitrogen.

In general, each of these steps was first thoroughly studied individually and then worked into the regular procedure at the most suitable point, conditions being selected with an eye to maintaining a reasonably good recovery of the enterocrinin during the process of purification.

The products obtained by the procedure as outlined above are not strictly uniform, tenfold differences in potency being not uncommon. Aside from the variability in the raw material, the salting-out procedure is the most wasteful and unreliable step, but no other procedure or combination of procedures has been found which will satisfactorily carry out its functions.

Figure 1 shows the assay results for the principal fractions of a dog gut preparation. It will be noted that up to fraction 14 the procedure was a better preparation of secretin and vasodilatin than of enterocrinin. The last three steps, however, greatly increased the enterocrinin potency, with relatively small losses, and at the same time removed all but 1 per cent of the secretin and reduced the vasodilatin content to a point where it was not detectable even with doses many times those required for the enterocrinin assay. The threshold dose[3] of the preparation was reduced from 12 mgm. (fraction 2) to 36 micrograms (fraction 30).

In addition to the data given, all the discarded fractions were worked up and assayed, and about 80 per cent of the original enterocrinin activity accounted for.

In this preparation about 70 per cent of the enterocrinin was lost during the

[3] The threshold dose of an enterocrinin preparation may be defined as the dose required to elicit 10 drops (0.3 cc.) of extra intestinal secretion from an *average* dog under strictly specified conditions. (See accompanying paper.)

salting-out step while comparatively little secretin or vasodilatin was removed. In the next dog gut preparation, however, this step lost about the same amount of enterocrinin, but removed 98 per cent of the secretin and all the vasodilatins. The usual result lies somewhere between these two extremes. Similar variation is encountered in hog gut preparations, but there the final potency achieved is
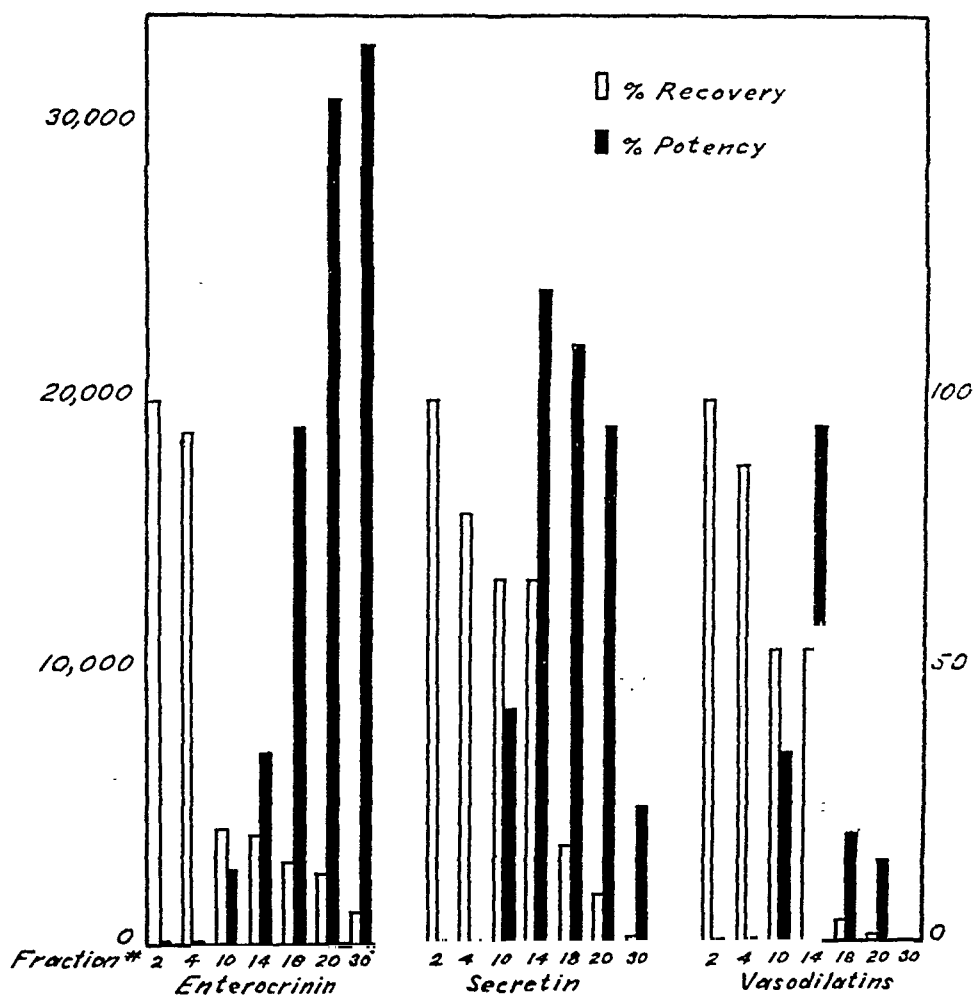
Fig. 1. Fractionation of an enterocrinin preparation. The open bars and the figures at the right indicate the percentage recovery in each of the fractions, of the three physiologically active substances named at the bottom of the groups, with the amount of each in fraction 2 taken as 100 per cent. The solid bars and the figures at the left represent the per cent potency of the three substances in each fraction, with their potency in fraction 2 taken as 100 per cent. The numeral below each pair of bars refers to the number of the fraction as given in the description of the fractionation procedure.

only about $\frac{1}{5}$ to $\frac{1}{10}$ of that obtainable from dog gut and the weight of the final fraction is proportionately larger (about 100 to 500 mgm. from 20 hog small intestines).

A number of other fractionation procedures have shown promise but have not been sufficiently studied to justify their incorporation into the regular procedure. Among this group are dialysis, electrophoresis, chromatographic adsorption, and

a number of precipitating agents. The most interesting results with dialysis experiments were obtained while using a cellophane membrane with aqueous acetic acid or aqueous methyl cellosolve as solvents. Under these conditions the porosity of the membrane may be controlled by adjusting the amount of water in the mixed solvent. Enterocrinin and secretin dialyzed at approximately the same rate under all conditions tested. In water solution most preparations are almost completely dialyzable, even through thick parchment. In the electrophoresis of crude extracts the enterocrinin moves toward the cathode ahead of the bulk of the preparation. The picrate derivative would appear to be the most suitable substance for chromatographic adsorption studies.

Attempts to purify enterocrinin by trichloracetic acid precipitation, isoelectric precipitation, electrodialysis[4], and molecular distillation have yielded very poor results.

The stability of enterocrinin has been found to vary widely according to the purity of the preparation used. In moderately pure preparations enterocrinin is relatively stable with respect to dilute acids and bases, heat, and oxidation. Very highly purified preparations, however, have lost a substantial proportion of their activity on being left open to the air in a $CaCl_2$ desiccator for a few months, and water solutions of these very potent preparations deteriorate within a few days or weeks even while stoppered and stored at 5°C.

No estimate can be given of the activity of pure enterocrinin, but it is certainly a powerful substance and a considerable amount of progress has been made toward its isolation. A check of the assay data concerning the most active preparation reported by Nasset (1938), using insofar as possible the new, improved methods of calculation, showed its threshold dose to be about 800 micrograms, as compared to the 80 microgram T.D. reported by Fink and Nasset (1941) and the 36 microgram T.D. reported in this paper.

SUMMARY

A detailed procedure is given for obtaining highly potent preparations of enterocrinin, the hormone which excites the secretory glands of the intestine. The procedure involves the extraction of intestines with slightly acidic 87 per cent alcohol, removal of the alcohol, salting out with NaCl, thorough drying of the salt cake between filter papers (to remove vasodilatins), fractionation with acetic acid, propylene glycol and methyl cellosolve, precipitation as the picrate, fractionation of the picrate with acetone, hot water, and acetic acid, and finally freeing of the hormone from the picric acid by precipitating it from a dry mixture of acetic and hydrochloric acids.

Data illustrative of the results obtainable are presented, and further methods of purifying the preparations are discussed.

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[4] We are deeply indebted to Prof. Einar Hammersten and Dr. Gunnar Agren of the Karolinska Institut (Stockholm) for furnishing their very fine electrodialysis apparatus for these tests.

The potency has been increased to over 300 times that of the crude extracts, so that as little as 36 micrograms is sufficient to produce an accurately measurable response in a 20 kgm. dog of average responsiveness.

REFERENCES

FINK, R. M. AND E. S. NASSET. This Journal **133**: P276, 1941.
    This Journal **139**: 626, 1943.
NASSET, E. S. This Journal **121**: 481, 1938.

INFLUENCE OF ALIMENTATION ON THE REGENERATION OF PLASMA PROTEINS FOLLOWING A SINGLE SEVERE NON-FATAL HEMORRHAGE[1]

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In previous communications (1, 2) in this Journal, changes in the red cell volume and the plasma proteins following an experimental bleeding were described. It was shown that the acute hypoalbuminemia which follows serious blood loss is corrected slowly, whereas the hypoglobulinemia returns to normal more rapidly. In these experiments the animals were not starved. In this report an attempt was made to study the effect of fasting as well as of various types of alimentation on the course of the regeneration.

Since our last paper (2) a few more observations have been described. Brown et al. (3) reported that following hemorrhage in man of 440 to 880 cc., hemodilution is detectable in one-half hour and continues for about 24 hours. If the blood loss is from an operation where there is extensive cutting of tissues, the hemodilution continues for 3 to 4 days. Beattie and Collard (4, 5) in anesthetized cats following single hemorrhages (20 per cent blood volume) obtained evidence that plasma proteins move into the blood stream within 2 hours after the hemorrhage. Considerable variations were observed in these experiments which the authors assume could be explained as being dependent upon the state of plasma protein stores outside the circulation.

METHODS. Mongrel dogs weighing between 8 and 15 kilos were used. They were allowed water but no food during the 18 hours preceding the experiment. Without anesthesia, except for the local use of novocaine when needed, the femoral artery was cannulated and the dog rapidly bled 35 to 45 cc. per kilogram of his body weight. No effort was made to affect the rate of blood flow from the cannula, but the process rarely required more than 2 minutes, after which the cannula was removed and the femoral artery tied off. The dog was then placed in a cage and allowed to have water ad lib. Except for immediate replacement of Ringer's solution in one group, the animal received no therapy until an hour after hemorrhage and then on the same hour on succeeding days. Heparinized blood samples were taken from the jugular vein or from the femoral vein in the leg opposite the one used for bleeding. These samples were centrifuged for 30 minutes at 3000 revolutions per minute. The plasma was analyzed for non-protein nitrogen and total nitrogen. For the former Nessler's reagent was used, for the latter a titrimetric micro-Kjeldahl procedure, involving steam distillation of the ammonia into a boric acid solution. For fractional determination of albumin the method of Campbell and Hanna (6) was employed. A few dogs died shortly

after the hemorrhage, but in this report only those dogs surviving 72 hours are included.
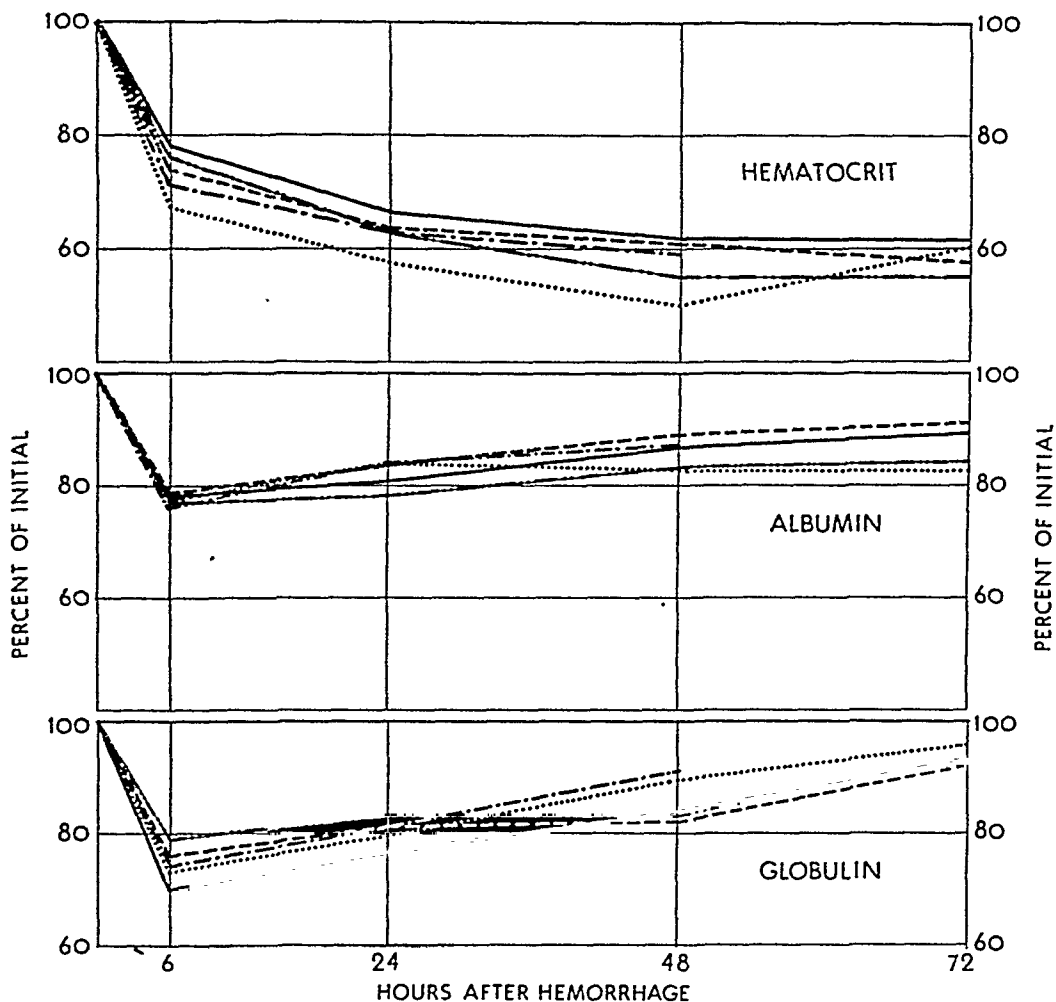
Fig. 1. Changes in hematocrit readings (red cell volume), plasma albumin and plasma globulin (expressed as percentages of initial which is represented as 100) during 72 hours following a single severe non-fatal hemorrhage, in five groups of experiments described in the text.

Symbols: group I, - - - -; group II,——; group III, ..., group IV, –··–··–; group V, –··–··–

Note the pronounced and continued hemodilution shown by the drop in red cell volume and the fall and slow rise in globulin and albumin. Note that the five curves are quite similar, indicating the relatively slight influence of the various dietary intakes. The rise in globulin in these experiments is less pronounced than in previous experiments.

Five groups of experiments were performed as follows:

Group I.   Ten dogs, 40 cc./kilo. hemorrhage; no repl; oral beef serum hydrolysate;[2] 75 cc./kilo. per day.

Group II. Ten dogs, 40 cc./kilo. hemorrhage, no repl; oral 10 per cent glucose in n.s.s. 75 cc./kilo per day

Group III. Five dogs, 40–45 cc./kilo. hemorrhage; no repl; water ad lib, no food

Group IV. Six dogs, 35 cc./kilo. hemorrhage; repl. with Ringer's sol.; reg. kennel diet

Group V. Six dogs, 35–40 cc./kilo. hemorrhage; no repl; oral Amigen;[2] 75 cc./kilo. per day

It will be observed that only dogs in group IV received the usual kennel ration; all of them, however, were allowed water ad lib. Those getting oral therapy received the solutions by stomach tube. Vomiting occurred very rarely and when it did the dog was re-fed immediately. The beef serum hydrolysate and the Amigen were both 10 per cent solutions plus 10 per cent dextrose. To these was also added vitamin B in the form of Labco 20 cc. per 1000 cc. of solution. All gavage feedings were given in doses of 75 cc. per kilo per day. Therapy was given daily for 72 hours, then the animals were sacrificed.

RESULTS AND COMMENT. The values for hematocrit and plasma proteins were calculated as a percentage of the initial (pre-hemorrhage) value, which was put at 100. It will be observed that (see figure) among these various groups of dogs there is remarkably little variation in the behavior of the hematocrit values and the plasma proteins. The hematocrit readings fall rather abruptly at first and then more gradually throughout the whole 72 hour period. At 6 hours after hemorrhage the lowest values for plasma albumin and globulin are to be found. From the 6th to the 72nd hour, plasma proteins show slow but steady regeneration, the globulin curve rising a little more sharply than the albumin curve.

In our previous study (2) it was inferred that the rising level of serum albumin and the constantly falling hematocrit values immediately following a single severe hemorrhage meant that the blood volume was being restored with albumin-containing fluid. In the present experiment it is interesting to note that this mechanism is not affected by the therapeutic measures used, i.e., food, beef serum hydrolysate, Amigen, 10 per cent glucose. Moreover, the dogs receiving nothing but water exhibit the same ability to compensate for this acute protein loss as do the other dogs.

These negative findings are significant because they show that the body compensates for the acute protein loss from a single severe non-fatal hemorrhage in a manner which is unaffected by the presence or absence of exogenous sources of protein or carbohydrate, excluding, of course, protein introduced intravenously as substitution therapy, i.e., plasma, whole blood or serum. Not described herein are experiments in which solutions of amino-acids and hydrolyzed protein were given intravenously following a single severe non-fatal hemorrhage; the same negative results were observed as with oral administration. On the other hand, we have obtained evidence (7) which indicates that dogs *succumbing* to *repeated hemorrhages* are beneficially influenced by such injections; it would seem that the compensatory mechanisms following hemorrhage differ with the manner and severity of the loss of blood.

It is interesting to note the results of group III. It is well known that a non-protein diet will result, in 2 to 3 weeks, in hypoalbuminemia in dogs. Apparently

a non-protein diet even after a severe hemorrhage has little effect on the serum proteins of dogs for as short a period as 72 hours. Other workers (8) have found that amino-acids are beneficial in protein regeneration following hemorrhage, but these studies extended over a much longer period of time (weeks and months) than the experiments reported in this paper.

CONCLUSIONS

The presence or absence of exogenous sources of protein or carbohydrate alimentation does not influence the rapidity with which the body compensates for the acute plasma protein loss during the first 72 hours following a single severe non-fatal hemorrhage.

REFERENCES

(1) ELMAN, R. This Journal 128: 332, 1940.
(2) ELMAN, R., C. LISCHER AND H. DAVEY. This Journal 138: 569, 1943.
(3) BROWN, G. L., J. A. R. MILES, J. M. VAUGHAN AND L. E. H. WHITBY. Brit. Med. J. 1: 99, 1942.
(4) BEATTIE, J. AND H. B. COLLARD. Brit. Med. J. p. 301, Sept. 12, 1942.
(5) BEATTIE, J. AND H. B. COLLARD. Brit. Med. J. p. 507, Oct. 31, 1942.
(6) CAMPBELL, W. R. AND M. I. HANNA. J. Biol. Chem. 119: 15, 1937.
(7) ELMAN, R. AND C. E. LISCHER. J. A. M. A. 121: 498, 1943.
(8) MADDEN, S. C. ET AL. J. Exper. Med. 77: 277, 1943.
     ROBSCHEIT-ROBBINS, F. S., MILLER, L. L. AND WHIPPLE, G. W., ibid. p. 375.

THE EFFECT OF CHOLINE AND CYSTINE ON THE SERUM PHOSPHATASE AND HEPATIC DYE CLEARANCE OF DOGS MAINTAINED ON DEFICIENT DIETS

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In a previous communication (1) it was reported that dogs fed a protein-deficient diet had a reduced liver function as evidenced by an increased serum phosphatase and a decreased hepatic dye clearance. The changes in liver function produced by a protein-deficient diet could be restored to normal by an adequate protein intake.

There are numerous reports in the literature (2, 3, 4, 5) which show that choline administration tends to reduce or prevent the accumulation of fat which otherwise occurs in the livers of rats fed a high-fat, low-protein diet. It has also been shown (6, 7, 8, 9) that cystine administration increases the percentage of fat in the livers of rats maintained on a high-fat, low-protein diet. However, there is no information in the literature concerning the effect of these two substances upon the function of the liver as measured by dye clearance or serum phosphatase. The present study was undertaken to supply such information. In addition, data were obtained on the effect of a choline deficiency without a superimposed protein deficiency on the dye clearance and serum phosphatase. The effect of feeding methionine to protein deficient dogs was observed in a few experiments.

METHODS. Healthy mongrel dogs or puppies were used in all the experiments. These were maintained for several weeks on a normal kennel diet, during which time control values for serum phosphatase and hepatic dye clearance were obtained. The dogs were then fed one of the deficient diets for varying lengths of time. The composition of the diets used is shown in the table on page 643.

Diet I is the protein-deficient diet used in the previous study. Diets II and III are similar to the low-choline, peanut meal diet described by Engle (10). In II the B-complex was supplied by the six per cent yeast in the basal mixture. In III the crystalline vitamins were used. The peanut meal[1] was percolated with 65 per cent alcohol for twenty-four hours and then extracted five times with boiling 95 per cent alcohol. The casein was extracted three times with the same solvent.

With only a few exceptions the serum phosphorus, phosphatase and hepatic dye clearance were determined at two week intervals. Bodansky's method (11) was used for phosphatase and the Rose Bengal test of Stowe, Delprat and Weeks (12) with the modification described in the previous publication (1), was used to determine hepatic dye clearance. The dogs were weighed weekly. Total liver lipids were determined in duplicate on the whole fresh liver, using the method of Best, Channon and Ridout (13).

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[1] Purchased from Darling Co., Chicago, Ill.

	DIET I	DIET II	DIET III
Lard................	33%	25%	27%
Sucrose............	55%	29%	31%
Salt mix..........	2%	4%	4%
Cellophane........	5%	0%	0%
B-complex........	Yeast 5%	Yeast 6%	Cryst. vitamins*
Protein...........	0%	Peanut meal 30%	Peanut meal 30%
		Casein 6%	Casein 6%

* The crystalline B-vitamins used were[2]: B_1, B_2, B_6, and calcium pantothenate (1 mgm./100 gram of diet), nicotinic acid, p-amino benzoic acid and inositol (10 mgm./100 gram of diet). One drop of Meade Johnson's percomorph oil was added to each 100 grams of diet. This supplied approximately 50 I.U. of vitamin D/kgm. and 350 I.U. of vitamin A/kgm. daily to adult dogs and double that amount to puppies.

The casein and peanut meal were extracted as described below.

The yeast used was Annheuser Busch Strain K.

All the diets were fed at a level of forty calories per pound of body weight for adult dogs and eighty calories per pound for puppies.

RESULTS. *Group I.* The effect of choline or cystine administration when fed at the beginning of a depletion period upon the serum phosphatase and dye clearance of adult dogs maintained on a diet practically free of protein (diet I).

a. Nine control animals were maintained on this diet and received no supplements. Five animals received diet I plus one-half gram of choline chloride daily in capsule form and six animals received diet I plus one gram of cystine daily throughout the experimental period. One animal was fed diet I, and given one gram of methionine daily for six weeks. At the end of that time the methionine was withdrawn and one-half gram of choline chloride was fed for two weeks.

b. Other dogs were fed choline or cystine supplements after they had been on diet I for varying lengths of time and had already exhibited increased serum phosphatase values and decreased rates of dye clearance. Several animals were fed $\frac{1}{2}$ or 1 gram of methionine daily after a period of protein depletion.

The differences in the serum phosphatase and hepatic dye clearance which were found in the twenty dogs in group I (a) with or without choline and cystine supplements are shown in table 1-a. The choline or cystine supplements which the treated animals in this group received were fed from the beginning of the depletion. The effect of choline during the first eight weeks is clearly seen when the serum phosphatase and hepatic dye clearance of this group are compared with the untreated group. At the end of this period the choline group's dye clearance had dropped from an original average of 104 ± 3.7 per cent of normal to 95 ± 5.8 per cent of normal. This is a relatively slight change. At the same time the untreated group's dye clearance had decreased from an original average of 99 ± 1.6 per cent of normal to 69 ± 6.2 per cent of normal. The

choline group's serum phosphatase increased only 5.8 units while the untreated group's increased 16.6 ± 3.5 units.

The effect of cystine is best seen during the early weeks of the depletion. These animals exhibited a decrease in dye clearance during the first two weeks

TABLE 1-a

DOG	SERUM PHOSPHATASE					HEPATIC DYE CLEARANCE				
	Weeks									
	0	2	4	6	8	0	2	4	6	8
Diet I. Protein-free,* no supplements										
						per cent	*per cent*	*per cent*	*per cent*	*per cent*
C-1	1.0	3.5	7.5	9.8	17.3	106	109	78	82	86
C-31	1.2	4.1	5.8	8.7	10.9	95	80	76	77	78
C-32	1.2	5.8	8.7	10.9	12.2	103	97	87	85	90
8-E	2.1	6.6	14.8	17.6	32.6	102	93	86	80	50
9-E	1.3	2.4	4.6	5.9	5.7	100	100	87	77	76
10-E	1.3	11.3	14.8	15.0	32.7	92	67	69	87	53
12-E	2.0	7.0	21.1	30.8		100	86	63	48	
31	0.5	2.4	3.6	7.2	12.8	98	79	68	89	78
33	1.1	22.1	18.8	20.7	18.8	93	50	64	51	41
Average....	1.3	7.2	11.0	14.1	17.9	99	85	75	75	69
Diet I. Protein free; one gram of cystine daily										
17-S	1.0	13.1	30.0	23.1	38.9	118	69	67	69	50
20-S	1.7	19.9	30.5	23.1	46.8	114	69	60	54	43
21-S	1.1	10.2	29.6	20.5	27.8	126	76	62	69	59
22-S	1.4	13.6	27.1	22.7	32.2	130	102	88	41	83
23-S	1.0	4.1	9.3	9.6	12.7	102	80	67	61	65
30	1.9	7.4	21.4	10.4	13.0	108	72	79	61	72
Average....	1.4	11.4	24.7	18.2	28.6	116	78	71	59	62
Diet I. Protein free; one-half gram choline chloride daily										
10-S	3.5	4.2	6.6	6.5	8.4	106	114	90	89	77
14-S	0.1	3.5	4.0	6.2	4.7	90	109	118	96	99
15-S	1.8	6.2	6.3	7.5	9.1	109	103	84	103	105
16-S	2.5	7.4	11.8	12.1	12.2	111	110	102	111	108
34	0.6	2.2	1.5	2.5	3.0	102	106	100	79	86
Average....	1.7	4.7	6.0	7.0	7.5	104	108	99	96	95

* Except for the protein in the dried brewer's yeast.

from 116 ± 4.1 per cent to 78 ± 6.0 per cent.   This decrease of 38 per cent is significantly greater than the two week decrease seen in the untreated animals which went from 99 ± 1.6 per cent to 85 ± 6.0 per cent, a decrease less than one half that seen in the cystine animals.   At the end of eight weeks the cystine

animals had decreased 54 per cent as compared to the 30 per cent decrease found in the untreated dogs. The serum phosphatase of the cystine animals was 28.6 ± 5.7 units in contrast to the 17.9 ± 3.5 units found in the untreated group. In comparing the cystine and untreated groups it will be noted that the differences between them are most pronounced early in the experiment. The

TABLE 1-b

Protein-deficient dogs

DOG	LIVER FAT	SURVIVAL	FINAL SERUM PHOSPHATASE	FINAL HEPATIC DYE CLEARANCE	WEIGHT CHANGE
			No supplements		
	per cent	*weeks*		*per cent*	*lbs.*
C-1	12.0	14	36.2	50	−5
C-31	6.7	16	8.8	61	−3¾
C-32	14.4	12	35.0	41	−7
31	8.2	10	26.9	46	−6
33	27.7	12	31.6	40	−5
Average......	13.8 ±2.7	12.8	27.7	48±	
			Choline supplement		
10-S	7.7	10	13.5	60	−1½
14-S	19.0	18	9.4	49	−6½
15-S	11.6	14	6.3	70	−7½
16-S	6.1	14	7.9	78	−6
34	5.4	10	4.3	107	−½
Average......	10.0 ±2.5	13.2	8.3	73	
			Cystine supplement		
17-S	15.7	10	32.3	59	−5½
20-S	19.5	12	7.4	40	−5½
21-S	11.2	12	20.3	54	+½
22-S	14.7	12	26.7	27	−½
23-S	7.0	16*	9.9	53	+1
30	10.5	10	10.9	70	−2½
Average......	13.1 ±1.8	12.0	17.9	51	

* Approximated.

cystine animals continue to exhibit decreases in dye clearance and increases in phosphatase, but the changes occur at a slower rate and eventually these values fall in the same range as the untreated animals whose values in the meantime have continued to drop.

Although all the animals survived longer than eight weeks this period of time was selected for the comparisons because soon after some of the animals developed varying degrees of anorexia. Serum phosphatase, liver lipids, and dye clearance

in dogs are all known to be affected by starvation (14, 15), consequently any values obtained after anorexia has developed become difficult to interpret. However, in order to obtain data on survival time all dogs (with the exception of four control dogs which were used to test other dietary supplements) were continued on the diet until they had developed practically complete anorexia. It was found that although the choline animals appeared better during the first eight weeks on the basis of the test used and also as judged by their appearance, they deteriorated rapidly after the eighth week and on the whole did not survive any longer than the control group. All three groups began decreasing their food intake about the same time. The average survival time of all the groups was between twelve and thirteen weeks. One of the factors which may have increased the variations among the individual animals in regard to survival time is coprophagy.

No control animals receiving adequate protein are included in this study because data on this aspect of the problem were presented previously (1) and gave adequate proof that diet I supplemented with 25 per cent casein protected dogs for as long as 30 weeks. No decreases in hepatic dye clearance were found, serum phosphatase remained normal, and liver lipids were low.

Since these experiments were planned primarily to study the changes in the phosphatase and dye clearance, it was impossible to control them in such a way as to make a satisfactory comparison of the liver fats in these animals. Nevertheless, the values found for liver lipids at autopsy are given in table 1-b along with survival time, final serum, phosphatase, dye clearance values, and weight changes. None of the differences between the cystine and untreated groups are at this time statistically significant. The choline group has significantly higher dye clearance and lower serum phosphatase but the liver lipids are not significantly lower.

The various experiments where cystine, choline or methionine supplements were fed after the animal had already been depleted are shown in table 2. The length of time the animal was maintained on diet I before the supplement was fed varied since the intention was to get a significant elevation in serum phosphatase and a decrease in dye clearance before giving one of the supplements. When choline was fed to these animals the serum phosphatase generally decreased and the dye clearance increased. Cystine usually had the opposite effect, causing a further rise in phosphatase and decrease in dye clearance. On one occasion the removal of the previously added cystine was followed by a large decrease in phosphatase, but no change in dye clearance, while in another instance its withdrawal was followed by an increase in dye clearance and only a slight decrease in phosphatase.

The depleted animals which were fed methionine gave variable results. In some cases this amino acid had the same effect as choline, in other cases it had no effect. The amount fed (one-half or one gram daily) was limited by the availability of the methionine and it seems reasonable to suppose that larger amounts fed for the same or greater periods of time would produce positive results. One animal[3] which received one gram of methionine daily from the

[3] Not shown in table 2.

beginning of a depletion responded in the same manner as a typical protein-deficient animal. At the end of six weeks his phosphatase was 17.3 units and his dye clearance had decreased from 111 per cent to 68 per cent. At this time the methionine was withdrawn and one-half gram of choline was fed. In one week the phosphatase had dropped to 5.2 units and the dye clearance had risen

TABLE 2

DOG	DAILY SUPPLEMENT TO DIET I	DAYS	PHOSPHATASE		DYE CLEARANCE	
			Start	End	Start	End
The effect of choline on dogs already depleted of protein						
					per cent	per cent
5-D	½ gram choline	14	35.8	14.7		
1-E	½ gram choline	21	7.2	3.8		
2-E	½ gram choline	14	32.6	12.7		
3-E	¼ gram choline	14	21.5	14.8	79	95
4-E	¼ gram choline	14	32.0	17.6		
8-E	½ gram choline	14	23.5	21.1	56	76
10-E	½ gram choline	14	17.9	25.2	57	73
12-E	½ gram choline	14	22.1	21.7	61	70
The effect of cystine on dogs already depleted of protein						
5-E	1 gram cystine	14	49.4	83.2	71	31
5-E	Cystine removed	14	83.2	44.9	31	34
9-E	1 gram cystine	14	5.2	15.9	63	33
9-E	Cystine removed	28	15.9	14.7	33	67
11-C	1 gram cystine	14	6.8	27.7	100	68
15-C	1 gram cystine	14	12.7	24.7	97	64
The effect of methionine on dogs already depleted of protein						
5-D	1 gram methionine	14	26.9	14.7	68	64
5-D	½ gram methionine (immed. after above)	14	14.7	15.6	64	82
5-D	Methionine removed	14	15.6	31.4	82	66
33	½ gram methionine	14	12.7	18.8	51	40
33	Methionine removed	14	18.8	31.6	40	53
11-C	½ gram methionine	14	30.1	38.5	61	58
11-C	Methionine removed	14	38.5	30.6	58	39

to 90 per cent of normal. In two more weeks the animal had become moribund and was sacrificed. Nine per cent fat was found in his liver.

Group II. The effect of choline upon the serum phosphatase and hepatic dye clearance of puppies fed a diet containing adequate amounts of protein but low in methionine and choline.

A group of six litter-mate puppies was fed diet II. Three of these were given one-half gram of choline chloride daily. The others did not receive this supplement. Five of the animals developed pneumonia after four weeks on the diet,

but since the changes which occurred during the first two weeks showed significant differences they will be considered. The results obtained are shown in table 3. The definite differences obtained after two weeks on the diet demonstrate how rapidly changes occur and the sensitivity of the tests used. During

TABLE 3

Six litter-mate puppies. Peanut meal, choline-deficient diet

DOG	SUPPLEMENT	0 DAYS			14 DAYS		
		Weight	Serum phosphatase	Hepatic dye clearance	Weight	Serum phosphatase	Hepatic dye clearance
		lbs.		*per cent*	*lbs.*		*per cent*
79P	None	$7\frac{3}{4}$	5.0	112	8	34.6	78
81P	None	$7\frac{1}{4}$	4.1	118	$6\frac{3}{4}$	16.8	66
82P	None	$7\frac{3}{4}$	5.8	123	$8\frac{1}{4}$	51.2	72
83P	Choline*	$6\frac{3}{4}$	5.2	118	$7\frac{1}{4}$	6.8	98
84P	Choline*	$8\frac{1}{2}$	4.9	120	$9\frac{1}{2}$	10.2	95
85P	Choline*	$7\frac{1}{2}$	5.4	123	$8\frac{1}{4}$	7.8	89

* 0.50 gram choline chloride daily.

TABLE 4

Three litter-mate puppies. Peanut meal, choline-deficient diet

DAYS	DOG 60P			DOG 62P			DOG 64P		
	Diet II—no choline			Diet III—no choline			Diet III—$\frac{1}{2}$ g. choline daily		
	Weight	Serum pt.	Hepatic clearance	Weight	Serum pt.	Hepatic clearance	Weight	Serum pt.	Hepatic clearance
			per cent			*per cent*			*per cent*
0	10	4.9	141	$10\frac{1}{2}$	3.4	134	$10\frac{1}{2}$	5.6	126
14	$10\frac{1}{2}$	12.4	134	10	35.6	115	$11\frac{1}{2}$	17.1	100
28	11	31.5	85	$9\frac{1}{4}$	24.4	84	$12\frac{1}{2}$	9.6	116
49	$11\frac{1}{2}$	30.1	88	Died. Liver fat 16.2%			15	9.7	96
63	$11\frac{1}{2}$	30.1	88				15	9.7	105
	Changed to Diet III						Food allowance reduced to equal amt. which 60P ate		
77	11		82				15		104
84	$10\frac{1}{2}$	33.0	50				$14\frac{1}{2}$		
	Sacrificed. Liver fat 30.0%						Sacrificed. Liver fat 4.3%		

this two week period the animals receiving choline gained an average of three-fourths of a pound, or about 10 per cent of their original weight. The deficient puppies averaged no gain in weight. All of them consumed their diet completely during these two weeks. One of the deficient puppies did not become sick when the others did and was continued on the diet for eleven weeks. At the end of this time he was emaciated and inactive. His food consumption had

gradually decreased from one hundred and fifty grams daily the first three weeks to about thirty or forty grams daily at eleven weeks. He had lost about twenty-five per cent of his original weight, had a serum phosphatase of 45.8 units and a dye clearance 67 per cent of normal. One-half gram daily of choline chloride was then administered orally. During the following seven days his food consumption increased until he was eating two hundred grams daily. His phosphatase dropped to 19.8 units and in another week was down to 4.0 units. His hepatic dye clearance increased to 97 per cent of normal in seven days and to 107 per cent in fourteen days. By the end of three weeks of choline administration he was eating 300 grams daily, had gained four pounds and was healthy and active. Choline was then removed and ten days later he suddenly became ill and died. There was 19.0 per cent fat in his liver.

In table 4 is presented the data obtained on three other litter-mate puppies maintained on diets II and III. Here again the choline-deficient animals had a higher serum phosphatase (24.4 and 33.0 units), and a lower hepatic dye clearance than did the animal which received choline. The choline-fed animal had only 4.3 per cent fat in his liver while the deficient animals had 16.2 and 30.0 per cent respectively.

DISCUSSION. It is apparent from the foregoing data that dogs receiving a high-fat, low-protein diet have a progressive impairment in the rate of removal of dye from the blood stream. The elevation of phosphatase is also progressive as the protein deficiency becomes more advanced. The anorexia which invariably develops in these animals may temporarily result in an increase in dye clearance and a decrease in phosphatase. The significance of the changes observed in the protein-deficient animals have been discussed previously (1).

The data presented in tables 1 and 2 indicate clearly that choline definitely aids in the maintenance of the liver function of the protein-deficient dog. That the beneficial effect of choline is temporary is also indicated by the eventual outcome of the experiments. Although at autopsy the liver lipids are in a slightly lower range than the untreated group and the dye clearance and serum phosphatase are closer to normal, the dogs had lost weight, developed anorexia and become moribund about the same time as the untreated animals. The animals in both groups had been practically devoid of dietary protein for more than ten weeks; and although the choline supplement may have prevented the infiltration of fat into the liver during the first eight weeks it could not prevent the loss of tissue protein from occurring during that time. This extensive loss of tissue protein results in numerous changes within the body which choline could not be expected to prevent. These changes and the anorexia associated with them cause the death of the animal, but should not be construed as casting doubt upon the effectiveness of choline during the earlier part of the experiment.

The results obtained on diets II and III indicate that a choline-deficiency in itself without a superimposed protein deficiency will cause a marked impairment in liver function in the normal puppy. In this case, however, oral choline supplements make the diet adequate and result in normal dye clearance and serum phosphatase. Schaefer et al have reported (16) that choline is essential for

the growing dog. The elevation in serum phosphatase which results from a choline deficiency in the puppy is very marked and rapid. The return to normal values which occurs when choline is fed is equally rapid. The data presented do not permit any conclusions as to the mechanism by which choline preserves liver function.

It is likewise apparent from the data that cystine early in the depletion increases the magnitude of the changes in dye clearance and serum phosphatase seen in the protein-deficient dog. The mechanism of the action of cystine is unknown. Cystine in large doses has been reported (17) to have a toxic effect upon the kidney. Griffith (5) has explained the action of cystine in accentuating the effect of a choline deficiency in growing rats as due to the acceleration in growth caused by cystine. This explanation is hardly applicable to the protein-deficient adult dog. It may be that the impaired liver function resulting from the protein deficiency impairs the means of utilization of cystine and thereby permits it to accumulate to the point of becoming toxic.

The experiments with methionine are inconclusive but indicate that on the weight basis this substance is less effective than choline in compensating for the protein deficiency.[4] This result is as one might expect if the methionine were serving as a source of methyl groups.

There does not appear to be a good correlation between the terminal fat content of a liver and its function as measured by dye clearance. Livers with a high function usually have a low fat content, but there are numerous instances of a low function and a low fat content of the liver. However, in such instances, histological examination frequently suggests other explanations for decreased dye excretion by the liver. This aspect of the problem will be considered in a later report.

SUMMARY

1. The decrease in dye clearance and the elevation of serum phosphatase caused by a protein-deficient diet are largely prevented in adult dogs for six to eight weeks by the daily ingestion of 0.5 gram of choline chloride. The effectiveness of choline was demonstrable only during the early weeks of the protein deficiency.

2. The changes in dye clearance and serum phosphatase caused by the protein-deficient diet are increased by the daily ingestion of one gram of cystine.

3. Choline-deficient puppies maintained on a diet low in methionine rapidly develop a marked increase in serum phosphatase and a decrease in dye clearance. Oral choline supplements will prevent or reverse these manifestations of impaired liver function.

REFERENCES

(1) Hough, V. H. and S. Freeman. This Journal 138: 184, 1942.
(2) Best, C. H. and M. E. Huntsman. J. Physiol. 75: 405, 1932.

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[4] Channon and collaborators (7) demonstrated that on a weight basis methionine is less effective than choline in preventing fatty livers in protein-deficient rats fed a high fat diet.

(3) BEST, C. H., M. E. HUNTSMAN, E. W. McHENRY AND J. H. RIDOUT.   J. Physiol. **86:** 315, 1936.
(4) MacLEAN, D. L., J. H. RIDOUT AND C. H. BEST. Brit. J. Exper. Path. **18:** 345, 1937.
(5) GRIFFITH, W. H. AND N. J. WADE.   J. Biol. Chem. **132:** 627, 1940.
(6) TUCKER, H. F. AND H. C. ECKSTEIN.   J. Biol. Chem. **121:** 479, 1937.
(7) CHANNON, H. J., M. C. MANIFOLD AND A. P. PLATT.   Biochem. J. **32:** 969, 1938.
(8) SINGAL, S. A. AND H. C. ECKSTEIN.   J. Biol. Chem. **140:** 27, 1941.
(9) WEBSTER, G. T.   J. Clin. Investigation **21:** 385, 1942.
(10) ENGEL, R. W. AND W. D. SALMON.   J. Nutrition **22:** 109, 1941.
(11) BODANSKY, A.   J. Biol. Chem. **101:** 93, 1933.
(12) STOWE, W. P., G. D. DELPRAT AND A. WEEKS.   Am. J. Clin. Path. **3:** 55, 1933.
(13) BEST, C. H,, H. J. CHANNON AND J. H. RIDOUT.   J. Physiol. **81:** 409, 1934.
(14) FREEMAN, S. AND C. J. FARMER.   Proc. Soc. Exper. Biol. and Med. **31:** 536, 1934.
(15) HOUGH, V. H., T. W. LI AND S. FREEMAN.   Unpublished data.
(16) SCHAEFER, A. E., J. M. McKIBBIN AND C. A. ELVEHJEM.   Proc. Soc. Exper. Biol. and Med. **47:** 365, 1941.
(17) EARLE, D. P., JR. AND J. VICTOR.   J. Exper. Med. **73:** 161, 1941.

THE POLYPHASIC ACTION CURRENTS OF THE MOTOR UNIT COMPLEX

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It has been well established that the motor unit, as defined by Liddell and Sherrington (14) is a "... motor neuron axon and its adjunct muscle fibers ...."

Gilson and Mills (12) point out that a motor unit can be identified by a "simple rhythmic discharge of recorded spikes of essentially constant height and form," and that "this criterion of single unit activity has been considered acceptable by previous workers."

In previous studies (7), we occasionally saw action potentials which were characterized by the above description except that the wave form was complex instead of simple. They consisted of a series of deflections of varying voltage and duration. This led to the question of whether these complex forms represent *a*, discharges from single fibers in a single unit; *b*, discharges from various fasciculi in a single unit, or *c*, discharges from several units which are perfectly synchronized.

A subject of equal importance is the functional organization of units in an intact muscle. Smoothness of muscle contraction would seem to require that a single unit spread its effect to a certain degree; it seems possible, therefore, that a unit may be distributed through the muscle rather than be restricted to a small area.

We have been unable to find reports concerning the temporal relationships between spike complexes picked up by two or more leads, or describing the area from which a unit can be recorded, although Lindsley (15) mentions that "the movement of the electrodes a millimeter one way or the other often completely eliminates the response of a unit."

Since these questions are of fundamental significance in the analysis of the electromyogram, particularly in its growing use as a means of studying normal and pathological activities of the central nervous system and of muscle itself, further studies of complex muscle action potentials have been made.

One hundred and thirty-one records were made from 23 men and 1 woman. The muscles studied were the deltoid, biceps brachii, triceps brachii, brachialis anticus, extensor communis digitorum and various layers of the spinal extensors. In addition, records from a former series dealing with recordings from the spinal extensors by concentric electrodes have been reviewed.

APPARATUS AND METHODS. Action potentials were amplified by dual balanced amplifiers and observations were made with a loud speaker and a cathode ray oscilloscope. Records were made on bromide paper by means of oscillographs.

Three types of electrodes were used: 1, concentric electrodes; 2, bare hypodermic needles. These are likely to pick up potentials from several motor units, and they apparently permit some loss of voltage by leakage or cancellation; 3, hypodermic needles covered to within 2 mm. of the tip with enamel. This is a compromise between the highly selective coaxial electrode and the bare needle. It is

most efficient when paired with a distant intradermal electrode. If two of these electrodes are placed together, they become selective, like the coaxial, and often fail to pick up activity which can be demonstrated by moving one away. These electrodes were used in nearly all of the experiments. They were sterilized by heat or by soaking in 70 per cent alcohol.

With the subject in a copper screen room, a suitable area was selected and the skin overlying the muscle was cleaned with 70 per cent alcohol. It was then anesthetized by an intradermal injection of 2 per cent procaine hydrochloride. The usual procedure was to insert one needle in the desired spot and a second 10–15 cm. distant, the second being inserted intradermally and used as an indifferent electrode. The needles were connected to the amplifier grids. A skin electrode covered with electrode paste served as a ground lead. The subject then created enough tension to activate a motor unit near the electrode in muscle. If a good single unit could not be brought in easily, the needle was moved until a suitable location was found. This electrode (no. 3) and the indifferent (no. 4) were connected to the A amplifier (A or upper tracing on the records) and used throughout the experiment as a reference electrode. Four other needles were then inserted around the central needle, those in the vertical plane (long axis of the muscle) were usually 2 cm. above and below the reference electrode; those in the transverse plane (right angles to the long axis) were usually 1 cm. from the reference electrode. The vertical electrodes were numbered 2, above, and 6, below, those in the transverse plane were 1, left, and 5, right.

Measurements of the voltage and time recorded photographically from action currents were made as follows: Voltage was measured by comparing the height of the spikes with those produced by known inputs. Time was measured from the peak of the main spike on the A channel, which was taken as a fixed point. Measurements of all deflections were taken with a micrometer ocular and graphs were made from those data. For each record, 10 spikes were measured and the data given below represent average values.

RESULTS. *The motor unit complex*. The muscle action potential of a single unit can be monophasic, diphasic, triphasic or polyphasic, as shown in figures 1 and 4, depending upon several factors. We have observed that all units in representative large muscles show distinctive patterns which vary in voltage and time with different electrode placements. The most common time intervals recorded ranged from less than 0.5 msec., where there is merely a hesitation or a notching, to 10–12 msec., where the two spikes are sharp and distinct.

The greatest disparity in time between spikes in one complex was seen in an experiment (fig. 4) where the time between the first and the last spikes of the complex was 22 msec. With a type 3 electrode, paired with an indifferent, picking up a single unit, a concentric electrode was inserted so that the tips of the type 3 electrode and the concentric were not more than 2 mm. apart. The first part of the complex was recorded simultaneously and with the same voltage on both. Twenty-two milliseconds later the concentric recorded an additional diphasic spike. The time between the first and last spikes thus recorded was constant in a series of photographs taken over a fifteen minute interval.

Augmentation and cancellation of the spike complex are demonstrated when two electrodes are in the field. When paired with the electrode out of the field
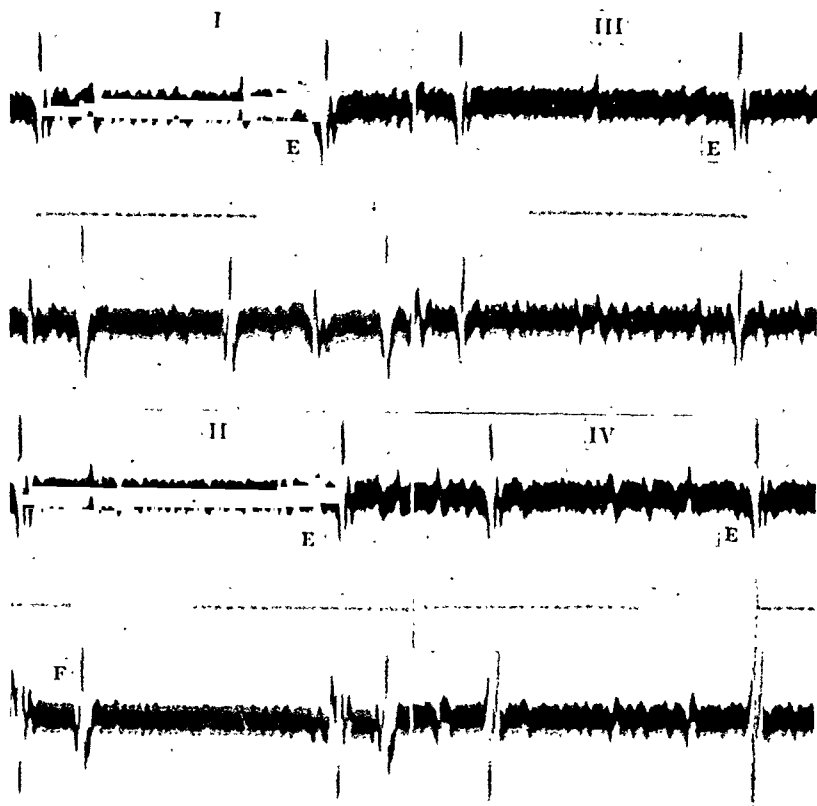
Fig. 1. Records taken with type 3 electrodes placed in the cross described above. The up swing is negative in all records. Time 0.25 sec.

Upper records: I–IV. electrodes 3 (in field) to 4 (out of field)

Lower records: I. Electrode 6 (2 cm. below 3 and in the field) to 4 (out of field).

Complex E—The single spike is nearly monophasic, an increase in amplification makes it triphasic, a decrease makes it purely monophasic. It occurs regularly 5.5 msec. before the main spike on the upper record.

II. Electrode 6 (2 cm. below 3 and in the field) to 3 (in field).

Complex E—The first negative spike on B is well ahead of the first negative spike on A and corresponds in time to the negative spike of E, lower record I. As 3 on B is in reversed position, in relation to the amplifiers, as contrasted to A, this large spike is obviously the pick-up at 3, while the early negative spike on B is from 6.

Complex F—The complex on the upper record is monophasic while on the lower it is polyphasic. The double negative spike and the notch in the second positive spike on B are examples of the complex wave form of the unit as described in the text.

III. Electrode 2 (2 cm. above 3 and in field) to 4 (out of field).

Complex E—The first and second negative spikes on B occur regularly 4.4 msec. and 6.6 msec. respectively after the large negative spike on the upper record. They correspond to the small spikes at the end of the complex on A in time but not in voltage.

IV. Electrode 2 (2 cm. above 3 and in field) to 3 (in field).

Complex E—The complex on B is a typical combination of the pick-up at 3 plus the pick-up on 2. 3 is in reversed position in relation to the amplifiers as contrasted to A.

each records a distinctive pattern. When they are inter-connected the temporal relation of the spikes of each discharge remains the same, with but minor varia-

tions. However, the voltages of the several spikes usually show marked change. In one experiment with two electrodes in the field, when the two were paired, a spike was recorded which appeared on neither when each was paired with an indifferent. In the same complex a spike, which was recorded with one electrode in the field, failed to appear when both electrodes in the field were paired as shown in figure 3.
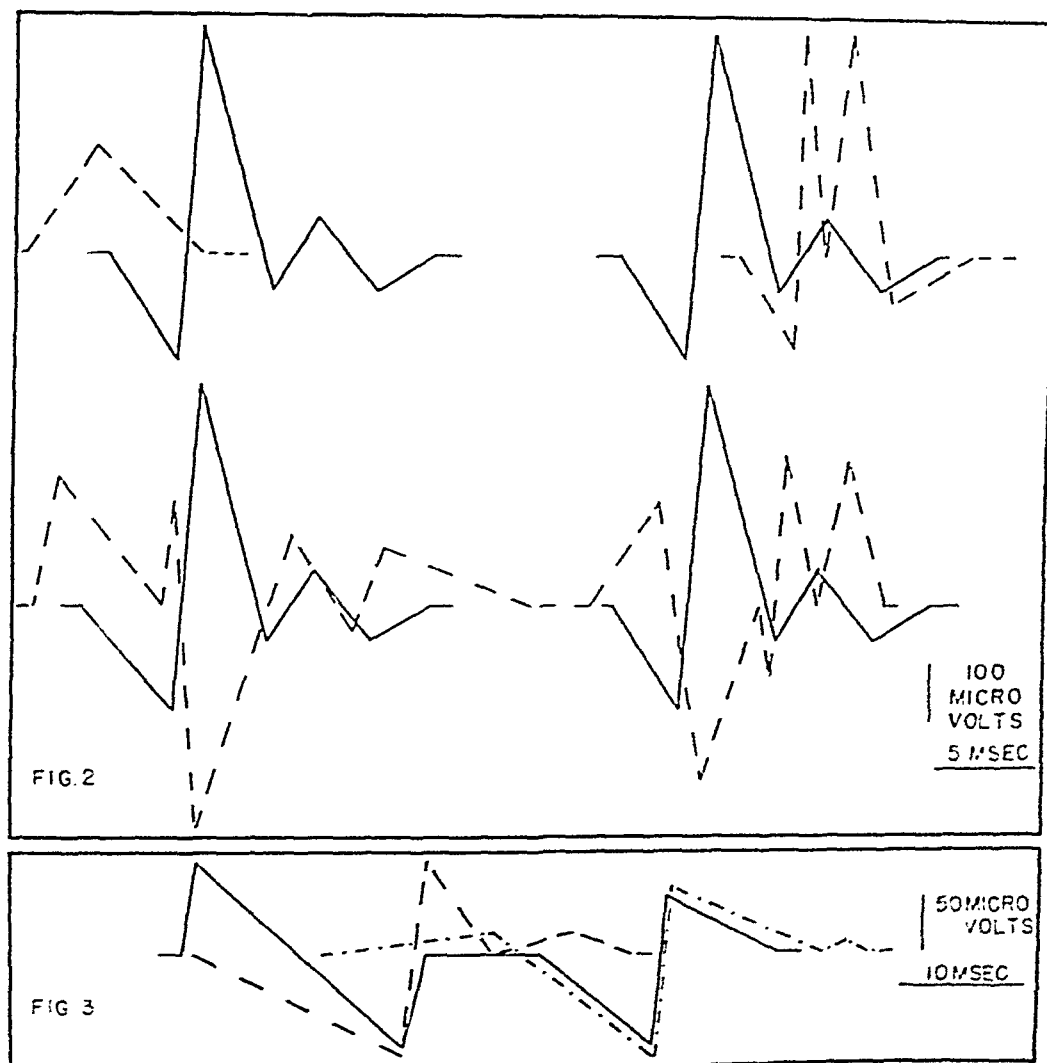
Fig. 2. Graphs representing the temporal and voltage relations of the E motor unit complex in figure 1: the upper left is I; lower left. II; upper right, III; lower right IV. The solid lines correspond to the upper records of figure 1 and the dashed lines to the lower records.

Fig. 3. Graphs representing the pick-up of two electrodes in a field. Solid line—electrodes interconnected. Broken lines—individual electrodes connected to indifferent electrodes.

Spatial delineation of the motor unit. Theoretically, it should be possible to ascertain the exact dimensions of the motor unit complex by moving the exploring electrodes back and forth until the area has been delimited. Practical difficulties, however, exist. The identity of the reference unit must be maintained at all times. If, through slight unavoidable electrode movement the pattern changes, the unit can no longer be identified positively as the original. In addition, after

numerous insertions and reinsertions of electrodes the subject frequently becomes tense and is unable to bring in the reference unit without having additional units contract.

In mapping the area from which a motor unit could be recorded, a contracting unit was located and electrodes were inserted as described. With the central reference electrode always on the A (upper) channel, all the others were connected in turn to the B (lower) channel. Some or all of the exploring electrodes picked up the activity. Electrodes in the field were moved outward, those out of the field were moved inward, until the limits were found.

In 52 placements 2 cm. from the reference electrode in the vertical plane, the unit was recorded 28 times and was not recorded 24 times. It was frequently recorded at distances less than 2 cm. and occasionally seen at greater distances.

Fig. 4. A—upper record—electrode 3 (in field) to electrode 4 (out of field).
B—lower record—concentric electrode, about 2 mm. from tip of 3.
Time 0.25 sec.
Electrode 3 and the concentric electrode both recorded a triphasic complex with an almost imperceptible notching of the main spike; 22 msec. after this complex, the concentric electrode recorded a diphasic complex which did not appear on the 3 to 4 combination.

In the transverse plane, in 38 placements 1 cm. from the reference electrode, the complex was recorded 21 times and was not recorded 17 times.

While these distances (2 cm. above and below and 1 cm. to each side of the reference electrode) were close to the outer boundaries in most instances, no distinct configuration of the motor unit has been found. Nor was it possible to determine the configuration from its voltage. In one experiment the highest voltage at any lead was 140 μv; the vertical area of the pick-up was 7 cm. and the transverse 6 cm. In another, the reference electrode voltage was 500 μv with no pick-up 1 cm. to either side and 2 cm. below. The voltage recorded on different placements ranged from 5 to 1000 microvolts as measured on the highest spike in the complex. These figures are relative, for electrode movement of less than a millimeter may change the voltage as much as 100 per cent.

The data given above refer only to the horizontal plane. All electrodes were inserted to equal depths, but a few trials showed that all parts of a complex were

not necessarily picked up at a uniform depth. Partial withdrawal of an electrode frequently changed its pick-up. In one experiment a needle inserted to full depth (25 mm.) recorded 240 μv. At 22 mm. it registered 300 μv; at 17, 60 μv; at 12, 50 μv. Even more striking variations were found in penetrating resistant fascial sheaths; on one side no activity might be found, whereas 1–2 mm. farther, on the other side of the sheath, a spike of 200–300 μv could be recorded.

Time of propagation. In 24 placements in the long axis of the muscle where an action current was recorded on 2 electrodes, measurements of the propagation time from one electrode to the other were made. The electrodes were from 0.5 cm. to 7.0 cm. apart. The measurements represent meters per second and were reached by dividing the space interval between the electrodes by the time required for the action current to travel from one electrode to the other. They are as follows: 1.3, 1.6, 2.2, 2.5, 2.6, 2.7, 3.1, 3.2, 3.4, 3.6, 3.6, 4.3, 4.3, 4.9, 5.2, 5.3, 5.9, 6.1, 7.1, 7.1, 7.7, 7.7, 10.9, 12.5.

The spike complex recorded by concentric electrodes. In a previous series where we were concerned only with the presence or absence of motor unit activity, concentric electrodes were used exclusively. A review of twenty-five single motor units recorded with these electrodes revealed that in 21 instances the spikes were di- or triphasic. In only 4 were they polyphasic. To verify this we made additional records with a concentric electrode. The wave form was altered by changing the position of the electrode (e.g., by rotation).

DISCUSSION. Three possible explanations for the polyphasic nature of the complexes recorded from motor units have been suggested. These are: *a*, action currents from single muscle fibers; *b*, action currents from several motor units firing synchronously; *c*, action currents from the fasciculi of a single motor unit.

Neither *a* nor *b* offers a sound explanation for polyphasic complexes. When the electrodes are compared in size with the muscle fibers, it is found that a large number of muscle fibers (diameter ca. 50 micra) would always be in contact with the electrode (diameter 450 micra). Therefore, if the individual muscle fibers were the source of the spikes, there would be more spikes than are actually found.

Many investigators have reported their observations on the frequency of motor units and there seems to be general agreement that two or more motor units do not fire synchronously except for extremely brief periods. To accept the theory that polyphasic action currents are due to a synchronization of motor units would require rejection of the concept of motor unit function which has been built upon the original observations of Adrian and Bronk (1, 2) and Denny-Brown (6).

Considerable evidence indicates that the polyphasic complex is due to *c*, action currents from the fasciculi of a single motor unit. Although little is known about the histology of the motor unit in large muscles, it has been shown to consist of a motor neuron and muscle fibers varying in number from 120 to 160 (9, 4, 16). Since muscle fibers are grouped into fasciculi containing about 20 fibers (3) and since the total number of fibers in a motor unit varies (11), it is

apparent that although the number cannot be fixed with certainty, there are at least several fasciculi in each unit.

Cooper (5) states that in the frog tenuissimus chains of fibers contract along the whole length of the muscle at all strengths of contraction.

The electromyographic data described above reinforce the idea that the motor unit is broken up into smaller components and that they are scattered. The spike complex usually registers as one or two strong action currents plus a number of weaker ones. We can infer from this that the electrode is near or in contact with one or two fasciculi and relatively distant from the rest.

Although there are no reports concerning the rate of conduction of impulses over the very small distal motor axon branches in the human neuromuscular system, the work of Erlanger and Gasser (10) permits the assumption that it is fairly slow, as is the velocity of the muscle action current. Katz and Kuffler (13) estimate the latter to be 2.–2.5 m./sec. in the frog sartorious, and Eccles and O'Connor (8) estimate rates of 2.85–4.8 m./sec. for cat soleus, 4 to 7 m./sec. for cat peroneus. Hence, the time difference of two action currents recorded on one electrode in the same unit can be accounted for by differences in the length and diameter of the involved axon branches and the spatial relation of the electrode to the fasciculus and its axon branch. In complex patterns when two leads record spikes in the same temporal relationship but with different voltages it is apparent that the spatial relation of the two to the fasciculi was similar except for slight differences in electrical contact.

There are interesting parallels between the polyphasic complexes we have seen and the polyphasic and notched complexes reported by Katz and Kuffler (13) in their studies of multiple motor innervation of the frog's sartorious muscle. In figure 3 of their paper, A2 and A3 show polyphasic wave forms representing the action potentials initiated first by nerve impulses over the pelvic branch, and a few milliseconds later by impulses from the tibial branch. In B2 and B3, with the pelvic branch cut, the first half of the complex wave fails to appear as the electrodes pick up one instead of two sets of action potentials.

Another interesting parallel is seen in figures 3A and B in a report of Lindsley (15). Although he did not discuss wave form, these figures show two negative and two positive spikes in clearly defined single units.

The demonstration of two or more spikes in a single motor unit implies that the unit is composed of discrete parts, and signifies that electromyographic studies must be interpreted on the basis of the functional capacity of the anterior horn cell with its motor neuron and of each of the muscle groups which it activates. It is apparent that both must be considered in any analysis of normal or pathological function of the final common path.

The present attempts to discover an exact pattern for the motor unit have been ineffective. No quantitative data are available concerning the distance an electrode can be located from a group of contracting fibers and still retain electrical contact. Our observations suggest that this distance is greater in the long axis of the muscle. This is perhaps due to greater conductivity of the fibers in that direction, but since fascial sheaths are in that plane, as insulators they doubt-

less restrict the pick-up in the transverse plane. Hence, these questions of conductivity and of interposed tissue must qualify the observations that the unit frequently occupies an area longer than its longest fiber and considerably wider than the combined diameters of its fibers. However, it has been apparent that an adequate sampling of an area 4 cm. square for muscle activity can be made by placing four type 3 electrodes, each paired with an indifferent, at the corners of a 1 cm. square.

In general it can be stated that the flow of action currents in a single unit is roughly parallel to the long axis of the muscle fibers and that it is progressive from one end of the unit to the other. We have never seen an instance where the action potentials of a single unit as recorded on electrodes 2 and 6 were both before or both after the complex as it was recorded on electrode 3.

The observations concerning propagation of the action current are qualified by the consideration of distance between active fibers and recording electrode, by the possibility that the two recording electrodes might be in different fasciculi (in one experiment the action current occurred simultaneously on 2 electrodes 2 cm. apart), and because precise electrode placement is not possible in unexposed muscle. Even with these qualifications, it seems safe to assume that the rate of propagation of the muscle action current is between 2.5 and 8.0 meters per second. These observations are in general agreement with previous reports (8, 13).

SUMMARY

1. Mono-, di-, tri- and polyphasic action currents from single motor units have been recorded, the pattern of the discharge being influenced by the type and location of the electrodes.

2. The area from which a motor unit can be recorded varies from 2 to 7 cm. vertically and 1 to 6 cm. horizontally; fascial sheaths probably act as dielectrics and limit the fields.

3. The evidence indicates that the components of the motor unit discharge originate in the several fasciculi which compose the motor unit.

REFERENCES

(1) ADRIAN, E. D. AND D. W. BRONK. J. Physiol. **66**: 81, 1928.
(2) ADRIAN, E. D. AND D. W. BRONK. J. Physiol. **67**: 119, 1929.
(3) Bailey's Textbook of Histology. 9th ed. William Wood & Co., 773 pp., 1936.
(4) CLARK, D. A. This Journal **96**: 296, 1931.
(5) COOPER, S. J. Physiol. **67**: 1, 1929.
(6) DENNY-BROWN, D. Proc. Roy. Soc. **B103**: 252, 1929.
(7) DENSLOW, J. S. AND C. C. HASSETT. J. Neurophysiol. **5**: 393, 1942.
(8) ECCLES, J. C. AND W. J. O'CONNOR. J. Physiol. **97**: 44, 1939.
(9) ECCLES, J. C. AND C. S. SHERRINGTON. Proc. Roy. Soc. **106B**: 326, 1930.

(10) ERLANGER, J. AND H. S. GASSER. Electrical signs of nervous activity. Univ. of Pennsylvania Press, 221 pp., 1937.

(11) FULTON, J. F. Physiology of the nervous system. Oxford Univ. Press, 675 pp., 1938.

(12) GILSON, A. S. AND W. B. MILLS. This Journal 133: 658, 1941.

(13) KATZ, B. AND S. W. KUFFLER. J. Neurophysiol. 4: 209, 1941.

(14) LIDDELL, E. G. T. AND C. S. SHERRINGTON. Proc. Roy. Soc. B97: 511, 1925.

(15) LINDSLEY, D. B. This Journal 114: 90, 1935.

(16) TERGAST, P. Arch. Micr. Anat. 9: 36, 1873.

THE REACTIVITY OF AUTONOMIC MEDULLARY CENTERS UNDER CONDITIONS OF RESTRICTED BRAIN CIRCULATION

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The measurement of blood flow through the brain under various experimental conditions has revealed an amazing degree of adaptability of the brain circulation. Rein and Schneider have shown that on clamping of one carotid artery, the blood flow in the remaining carotid is increased about one hundred per cent. Successive ligation of the contralateral carotid and of both vertebral arteries· increases the blood flow through the interna carotid more than twice. These reactions appear to be responsible for the remarkable fact that even the clamping of both carotids does not cause any permanent effect on the cerebral potentials of the cat (Bremer). Asenjo observed that only the clamping of both carotids and one vertebral artery definitely reduced the brain potentials. The reactivity of the autonomic centers in these conditions of restricted brain circulation has not yet been studied adequately. Since the vasomotor as well as the respiratory center reacts with great precision to slight alterations in the oxygen and carbon dioxide tension of the blood, it seemed of interest to investigate the reactivity of these centers when one or more of the main arteries are ligated temporarily.

METHODS. The experiments were carried out on 43 dogs anesthetized in most instances with 70–100 mgm./kgm. chloralosane intravenously. In some animals sodium amytal (55 mgm./kgm. intraperitoneally) was used. Blood pressure and respiratory rate were recorded in the usual manner. The respiratory minute volume was determined by means of a spirometer. The respiration was either spontaneous or artificial after a pneumothorax had been made. Gas mixtures ranging between 4.5 and 12.5 per cent oxygen and 3.2 and 15 per cent carbon dioxide were inhaled from Douglas bags.

RESULTS. I. *Observations on the effect of anoxia and hypercapnia on blood pressure and respiration of "denervated"[1] dogs under conditions of restricted brain circulation.* In order to study the reactivity of the vasomotor and respiratory centers to changes in the oxygen and carbon dioxide tension of the blood under the conditions of restricted brain circulation it was deemed advisable to eliminate the pressor and chemo-receptors which modify the reactivity of these centers. It was found in such preparations that the blood pressure response to inhalation of gas mixtures low in oxygen did not show any significant alteration in the fall of the blood pressure after one carotid artery or one or both vertebral arteries had been clamped. In the light of the experiments reported below, this suggests that no significant disturbance in brain circulation is induced under these conditions.

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[1] Denervation of the sino-aortic area.

If, however, both carotid arteries are ligated, the reactivity of the respiratory and vasomotor centers is altered significantly. Figure 1 shows that the respiratory response to carbon dioxide is definitely increased under these conditions, although the clamping of the carotids as such does not change the respiratory minute volume. Furthermore, in experiments involving the inhalation of gas mixtures low in oxygen it is found that the fall in blood pressure which occurs in the "denervated animal" in anoxia is greatly increased after bilateral clamping of the carotids (table 1). In the experiments involving the use of oxygen-nitrogen mixtures a constant respiratory volume was maintained by artificial
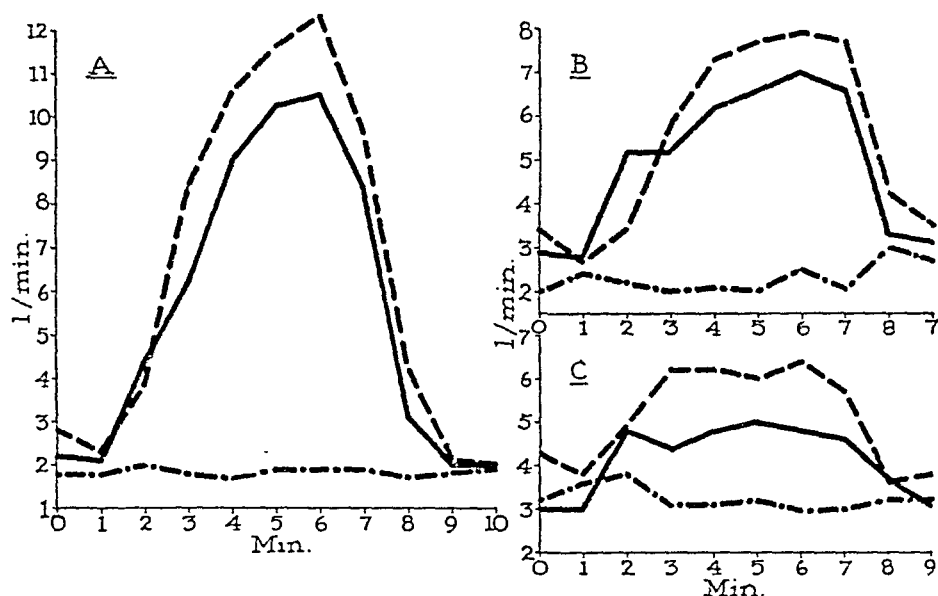
Fig. 1. A. Effect of 5 per cent CO_2 on the respiratory volume during clamping of both carotid arteries. Dog 38, 3/28/40, 9.1 kgm., 70 mgm./kgm. chloralosane intravenously. —·—·—, clamping of carotids between 1 and 9 while dog inhales air.————, inhalation of 5.4 per cent CO_2 from 1 to 6. ———— inhalation of 5.4 per cent CO_2 from 1 to 6 while carotids are clamped from 1 to 9.

B. Effect of 5.4 per cent CO_2 on the respiratory volume of a "denervated" dog while the carotids are clamped. Dog 44, 4/22/40, 10 kgm., 70 mgm./kgm. chloralosane intravenously.

C. The effect of 3.2 per cent CO_2 on the respiratory volume of a "denervated" dog while the carotids are clamped. Same dog as in figure 1, A.

respiration since it was known from the work of Gellhorn and Lambert that the diminution or failure of respiration after elimination of the carotid sinus nerves may secondarily cause a fall in blood pressure. Gellhorn and Lambert's interpretation that the fall in blood pressure occurring in denervated dogs under conditions of anoxia is due to a depression of the vasomotor center appears to be applicable to these experiments. The augmented fall in blood pressure in response to anoxia suggests that the vasomotor center is more susceptible to anoxia after the carotid arteries have been clamped than under normal conditions.

It is interesting to note that this reaction may be observed also when in an animal in which both vertebral arteries had been permanently ligated the carotid

arteries are clamped temporarily. It is then found that under conditions of anoxia the fall in blood pressure observed on inhalation of 5 per cent oxygen is greater after the two carotid arteries had been clamped.

Experiment 4 of table 1 illustrates the effect of 6.2 per cent oxygen on the blood pressure of a "denervated" dog before and after ligation of the carotid arteries under conditions of spontaneous respiration. Here, likewise, a greater fall in blood pressure is observed under conditions of restricted brain circulation. In view of our earlier studies this reaction may, at least in part, be due to more rapid failure in respiration after the carotids have been ligated.

From these experiments it seems to follow that ligation of one carotid artery or of both vertebral arteries is fully compensated and does not lead to any altera-

TABLE 1

Effect of restriction in brain circulation on the blood pressure response to anoxia in dogs with denervation of the sino-aortic area

NO.	GAS MIXTURE	DURATION OF ANOXIA	EFFECT OF ANOXIA ON BLOOD PRESSURE					
			Without clamping		With clamping		Without clamping	
					of both carotid arteries			
				Diff.		*Diff.*		*Diff.*
1	5% O$_2$	25 sec.	114– 96	18	120– 86	34		
					123– 90	33		
2	6.2% O$_2$	45 sec.	92– 73	19	104– 76	28	88– 72	16
					98– 74	24		
					95– 72	23	84– 74	7
3	4.5% O$_2$	60 sec.	130–115	15	170–115	55	120–100	20
4	6.2% O$_2$	5 min.	125– 85	40	145– 65	80	Spontaneous respiration	

(For experiments 1–3, the last column group is bracketed together with the note: Pneumothorax and artificial respiration)

tion in the reactivity of medullary centers when tested with gas mixtures low in oxygen or rich in carbon dioxide.

However, bilateral ligation of the carotid arteries leads to a disturbance in brain circulation which is revealed by the increased response of medullary centers to anoxia and carbon dioxide.

II. *Effect of restriction in brain circulation on the blood pressure and respiratory response to anoxia and carbon dioxide excess in normal and vagotomized animals.* Since ligation of the carotids of "denervated" dogs resulted in a greater depression of the vasomotor center to anoxia than was seen after restoration of the normal circulation, one might expect that in normal and vagotomized animals the pressure response to anoxia would be diminished under these conditions. However, the result is quite different. As figure 2 shows, ligation of the carotids greatly increases the pressure response to anoxia. Since anoxia depresses the vasomotor center more after the ligation of the carotids as shown by the experiments on "denervated" animals described in the preceding paragraphs, the

intensification of the pressure response must be due to other than central factors. Considering that in normal animals the ligation of the carotids greatly alters the reflexes mediated by the buffer nerves, it is probable that they are responsible for the alteration in the blood pressure response. The lowering of the pressure
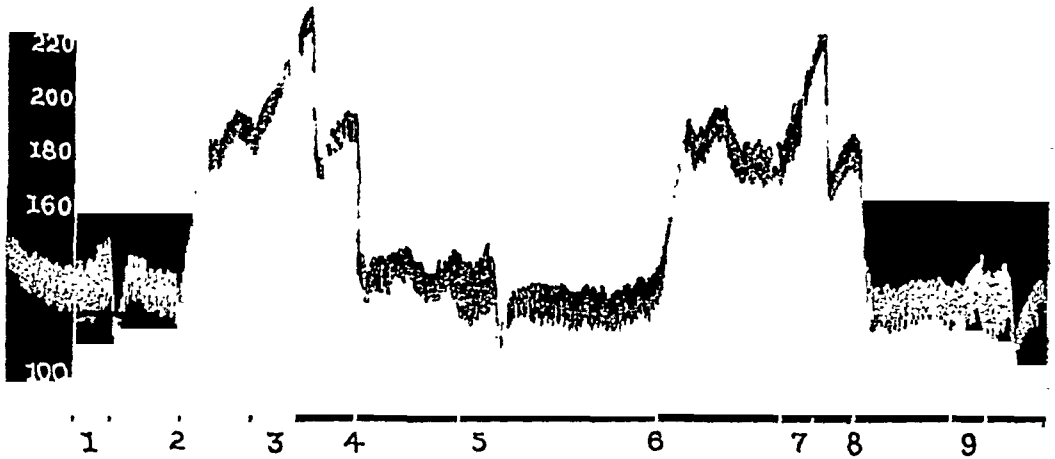
Fig. 2. The effect of 6.2 per cent O_2 inhaled for 1 minute on the blood pressure before and during restriction of brain circulation (clamping of both common carotids). Dog 9, 12/7/39, 8.2 kgm., chloralosane 100 mgm./kgm. intravenously. Bilateral vagotomy. *1, 5* and *9*: 6.2 per cent O_2 under control conditions; *3* and *7*: 6.2 per cent O_2 while both carotids are clamped (from 2 to 4 and 6 to 8 respectively).
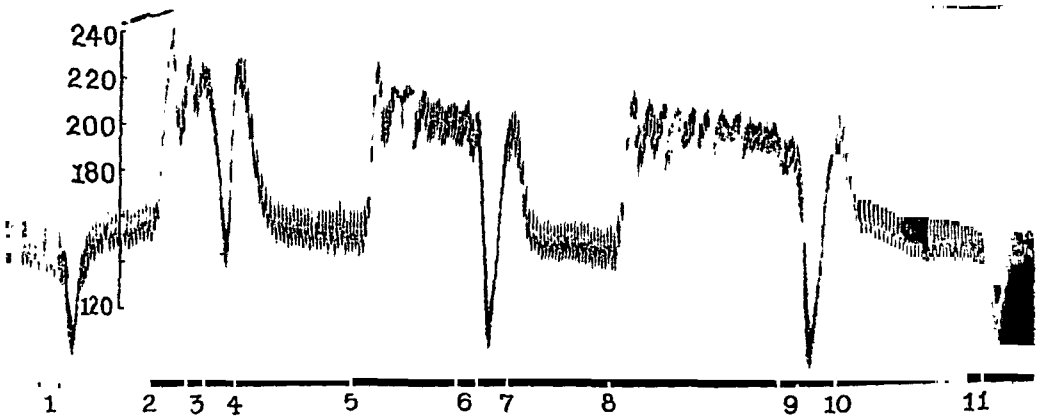
Fig. 3. Effect of 15 per cent CO_2 on blood pressure before and after clamping of both common carotids. Dog 29, 3/1/40, 7.6 kgm., 70 mgm./kgm. chloralosane intravenously. *1, 11*, 15 per cent CO_2 inhaled for 30 sec. under control conditions. *3, 6* and *9*, the same but carotids are clamped (from *2* to *4*, *5* to *7* and *8* to *10* respectively).

in the carotid sinuses which follows the ligation of the carotid arteries central to these structures is similar in its effect to that seen after removal of the pressure receptors. If this is the case, any factor which either raises or lowers the blood pressure should produce greater alterations in blood pressure after the carotid arteries had been clamped. It was, indeed, shown that the blood pressure rise following inhalation of carbon dioxide was also greatly exaggerated. It is of

interest to note that in several animals in which either anoxia or carbon dioxide caused a fall in blood pressure instead of a rise, this fall was greatly enhanced after the carotid arteries had been clamped (fig. 3).

It may be added that ligation either of one carotid artery or of both vertebral arteries did not alter the blood pressure response to anoxia.

Experiments on the effect of the inhalation of carbon dioxide under conditions of restricted brain circulation caused similar effects in normal and vagotomized dogs as have been described for animals deprived of the sino-aortic receptors (fig. 1). It was found that carbon dioxide had a greater effect on respiration after ligation of both carotid arteries. Since carbon dioxide stimulates the respiratory center as well as the chemoreceptors, it is not surprising to find that the reactivity of normal and "denervated" animals is similar. The increase in respiratory response to carbon dioxide after ligation of both carotid arteries varied considerably in different experiments but was distinct in all animals independent of the presence of the buffer nerves.

The increased level of the blood pressure together with the augmented blood pressure and respiratory response to anoxia and hypercapnia represents an adjustment reaction which tends to restore an adequate blood supply to the brain in spite of the elimination of both carotid arteries.[2]

CONCLUSIONS

The reactivity of the vasomotor and respiratory center under conditions of restricted brain circulation is determined by comparing the effects of anoxia and hypercapnia on blood pressure and respiration before and after ligation of one or more carotid and vertebral arteries.

It is found that neither ligation of one carotid or of one or both vertebrals alters the blood pressure response to anoxia.

Ligation of both carotids leads to an increased blood pressure response to gas mixtures low in oxygen in normal and vagotomized animals. After denervation of the sino-aortic area the blood pressure falls more rapidly during anoxia after both carotids had been ligated indicating an increased sensitivity of the vasomotor center to anoxia under conditions of restricted brain circulation.

The respiratory response to hypercapnia is increased after bilateral ligation of the carotids. This effect present in normal and vagotomized animals as well as in dogs in which the sino-aortic area had been denervated signifies an increased sensitivity of the respiratory center to hypercapnia.

REFERENCES

(1) REIN, H. Ergebn. d. Physiol. **32**: 28, 1931.
(2) SCHNEIDER, M. AND D. SCHNEIDER. Arch. f. exper. Path. u. Pharmakol. **175**: 606, 1934.
(3) BREMER, F. Compt. rend. Soc. biol. **118**: 1241, 1935.
(4) ASENJO, A. Zentralbl. f. Neurochir. **3**: 198, 1938.
(5) ASENJO, A. Zentralbl. f. Neurochir. **4**: 41, 1939.
(6) GELLHORN, E. AND E. H. LAMBERT. The vasomotor system in anoxia and asphyxia. Urbana, Ill., 1939.
(7) GELLHORN, E. Autonomic regulations. New York, 1943.

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[2] For further discussion cf. Gellhorn (1943).

ELECTROLYTE REDISTRIBUTION IN CAT HEART AND SKELETAL MUSCLE IN POTASSIUM POISONING

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Previous work in this and other laboratories has shown that both sodium and potassium traverse the membranes of living muscle cells under a variety of conditions (1, 2, 3, 4, 5, 6, 7). Within physiological limits muscle sodium and potassium may exchange for each other without disturbing the osmotic relationships in the cell or the ability of the cell to contract (8). In an experiment designed to explore some of the factors influencing the tolerance of rats to lethal doses of potassium chloride, Miller and Darrow (9) found the potassium of voluntary muscle above the normal range in all of the poisoned animals except those in which previous depletion of muscle potassium had been effected by a low potassium diet. Their report confirmed earlier observations (10) that there is a close correlation between the signs of potassium poisoning and the level of serum potassium, but in addition that poisoning can occur when skeletal muscle potassium is below the normal range.

The sensitivity of the heart to potassium poisoning provides a unique opportunity to examine the electrolyte concentration in both the heart and the serum at a time when functional changes resulting from the increase in potassium become critical. If the toxic effects of potassium depend upon its accumulation within the heart muscle fibers, this accumulation might be expected to bear some quantitative relationship to the onset of signs of poisoning. We have studied the distribution of the principal electrolytes in the heart, skeletal muscle, and serum of cats injected intraperitoneally with potassium chloride solutions, using an electrocardiograph and cardioscope[2] to detect the onset and follow the progress of potassium poisoning.

METHODS. The animals used were adult cats maintained for varying periods, usually a week or more, on a diet of canned salmon and milk. The animals were anesthetized with 37 mgm. per kilogram body weight of pentobarbital

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[1] On leave of absence from the Department of Physiology, Stanford University, California.

[2] Sanborn Cardiette and Cardioscope.

given intraperitoneally. Preparations were made for obtaining the standard electrocardiograph leads. After preliminary electrocardiograms were taken, control samples of arterial blood were drawn under oil from the exposed femoral artery. Potassium chloride was given by intraperitoneal injection of 0.5 molar solution in doses varying from 5 to 8 m.eq. per kilogram body weight. In six experiments a solution containing 16.0 grams per liter of sodium chloride, 4.4 grams per liter of sodium bicarbonate and 18.65 grams per liter of potassium chloride was used to supply sodium in slightly more than twice physiological concentration. In two experiments the animals were subjected to the removal of electrolytes by the intraperitoneal injection and subsequent removal of 100 cc. per kilogram body weight of 5.5 per cent glucose solution before the potassium solution was administered.

After the potassium solutions were injected, interval electrocardiograms and arterial blood samples were taken. Where electrocardiographic evidence of potassium poisoning was seen in the cardioscope, the changes were followed until no P waves could be made out, and broad, biphasic complexes indicated severe interference with conduction throughout the ventricle. A final blood sample was drawn, and the heart was removed. The interval between the appearance of diffuse block and the removal of the heart was, in most cases, less than 3 minutes. The heart was then freed of blood and most of the fat, placed in tared vessels and weighed. Skeletal muscle samples were removed from the hind legs and the wet weight obtained in the same manner. Total water was measured in each sample by drying to constant weight at 105°C. Determinations of sodium, potassium, chloride, phosphorus, nitrogen, and fat were made on each of the serum and tissue samples by methods cited in previous communications (1).

RESULTS. *The electrocardiographic evidence of potassium poisoning.* Our findings were essentially the same as those reported by Winkler, Hoff and Smith (10) in experiments on dogs receiving potassium chloride solutions in isotonic concentration intravenously. In our experiments the duration of the QRS complex remained unaltered until the serum potassium concentration rose to about 8.5 m.eq. per liter. Increases above this level brought about a progressive widening of the QRS. Figure 1 illustrates the relationship where the log of the QRS duration is plotted against the log of the serum concentration of potassium.

Analysis of the influence of potassium on the P-R interval shows that the critical concentration lies at about 11.0 m.eq. per liter. No progressive increase in the length of P-R could be detected as the critical level was approached. Beyond the critical serum concentration, P waves were no longer identifiable.

Rate of increase of potassium concentration. It was observed that considerable differences could occur from animal to animal in the length of time from injection of potassium to the disappearance of P waves. The animals showing early block tended to manifest this sign of potassium poisoning at lower serum concentrations than those in which the interval was longer. It was also noted that the response of the animals to potassium injected intraperitoneally did not bear a strict relation to the dose given. In general few animals receiving 5 m.eq. per kilogram body weight or less showed signs of poisoning, and all receiving as much as 7 m.eq. per kilogram developed heart block.

Data from our experiments furnish evidence that the absolute level of serum potassium is not the sole factor determining the influence of potassium on the heart as suggested by Winkler, Hoff and Smith (10). In an earlier series of experiments Winkler and Smith (11) report that animals tolerate the administration of very large amounts of potassium given intravenously if the rate of administration is slow. An increasing concentration of serum potassium required that the rate of absorption from the peritoneal cavity must exceed the rate of removal of potassium from the blood by all routes. Figure 2 illustrates the relationship between the rate of rise of serum potassium and the time required for block to appear.

If the level of serum potassium were the sole factor responsible for the onset of heart block in the poisoned animals, one should expect far greater uniformity in the measured serum potassium concentration at the time of heart block than was observed in our experiments. The concentrations of serum potassium found
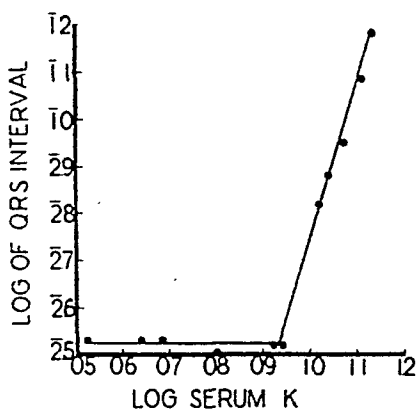
Fig. 1
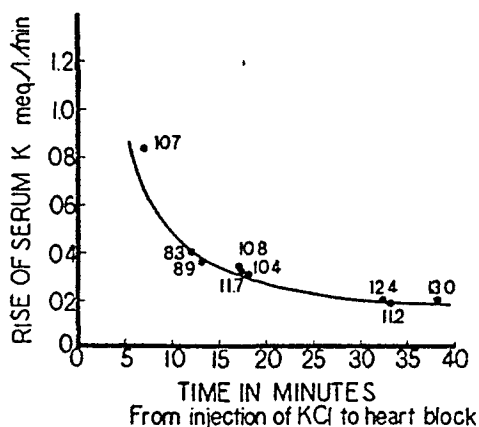
Fig. 2

Figs. 1 and 2. The numbers beside the points on the curve in figure 2 indicate the concentration of serum potassium at the time of heart block.

at the time of heart block ranged from 8.26 to 13.4 m.eq. per liter. One factor which may account for the appearance of block at relatively low serum potassium concentrations is a high rate of rise of serum potassium. In figure 2 the number beside each of the points on the graph indicates the serum potassium concentration at heart block. In general, early block is associated with rapid rate of rise of serum potassium rather than with the higher concentrations of potassium. This relationship indicates that the influence of potassium on the heart may be determined by the rate of change of potassium concentration as well as by the absolute concentration reached.

Electrolyte redistribution following potassium injection. In table 1 are presented the terminal serum concentrations of electrolytes and those found on analysis of heart and skeletal muscle. While the concentrations of electrolyte in the heart are not normal there is no clear association of the abnormalities with heart block.

Potassium. It will be noted that, per unit weight of tissue, the heart took

TABLE 1

NO. OF ANIMALS	TREATMENT	BLOCK	SERUM				HEART						MUSCLE					
			H₂O per liter	Cl	Na	K	H₂O per 100 gm. fat-free dry tissue	N	Cl	P	Na	K	H₂O per 100 gm. fat-free dry tissue	N	Cl	P	Na	K
			gm.	*m.eq. per liter*			*gm.*	*m.eq. per 100 gm. fat-free dry tissue*					*gm.*	*m.eq. per 100 gm. fat-free dry tissue*				
6	KCl 0.5 M	−	930.8 ±3.81	120.2 ±2.01	147.1 ±5.09	8.1 ±0.50	403.7 ±7.55	15.2 ±0.36	20.6 ±1.04	36.0 ±1.12	21.3 ±1.03	45.7 ±1.30	334.0 ±4.20	15.5 ±0.12	6.2 ±0.63	32.9 ±0.37	7.8 ±0.50	48.3 ±0.53
8	KCl 0.5 M	+	931.4 ±2.41	119.7 ±1.79	151.6 ±3.82	12.3 ±0.79	414.6 ±9.16	15.1 ±0.09	22.5 ±0.76	35.8 ±0.91	22.4 ±1.16	47.5 ±0.62	334.8 ±4.46	15.3 ±0.14	7.2 ±0.69	32.9 ±0.65	8.0 ±0.37	48.5 ±0.76
2	KCl + extra Na*	−	933.0	124.4	144.2	8.9	401.0	14.9	20.9	37.1	22.0	47.8	328.0	15.3	5.8	31.4	7.6	47.1
4	KCl + extra Na	+	928.8 ±1.39	130.1 ±1.42	156.2 ±1.21	11.9 ±1.76	370.5 ±16.0	14.8 ±0.42	22.7 ±2.03	35.0 ±2.38	20.5 ±2.68	48.7 ±2.80	327.0 ±7.20	15.2 ±0.13	6.8 ±0.83	32.6 ±0.15	8.8 ±0.65	46.5 ±1.30
1	Glucose, KCl†	−	926.0	119.6	132.6	9.3	425.0	15.1	21.8	38.4	20.5	49.6	344.0	15.4	6.8	33.0	8.4	50.8
2	Glucose, KCl	+	899.5	100.0	123.0	12.6	373.0	14.8	17.0	34.9	18.1	40.4	337.0	15.4	4.8	31.9	6.4	46.2
24	Normal controls	−	932.0 ±0.2	118.5 ±0.15	149.5 ±1.10	4.7 ±0.13	410.0 ±5.5	14.1 ±0.18	19.1 ±0.4	34.0 ±0.6	24.4 ±0.9	40.1 ±0.7	345.0 ±3.2	15.3 ±0.03	5.9 ±0.21	32.7 ±0.23	8.0 ±0.21	47.4 ±0.37

* Solution given intraperitoneally contained NaCl, 16.0 grams per liter, sodium bicarbonate 4.4 grams and potassium chloride 18.65.
† Injection intraperitoneally of 5 per cent glucose, 100 cc. per kgm. followed in 3 hours by the removal, by paracentesis, of a volume of fluid equal to the volume of 5 per cent glucose solution injected.   The KCl in 0.5 M solution was injected immediately after the paracentesis.

up far more potassium than skeletal muscle. In many cases the potassium of skeletal muscle was found to be lower than that of normal, untreated cats. This difference in potassium uptake may be only a result of the wide difference in circulatory minute volume through the two organs.

The heart muscle potassium was raised significantly in all animals receiving potassium intraperitoneally, except those deprived of extracellular phase electrolytes by the injection and subsequent removal of 5.5 per cent glucose solution. Normal cat heart muscle contains 40.1 ± 0.7 m.eq. per 100 grams of fat-free dry tissue. The experimental group had heart potassium values from 43 to 54 m.eq. per 100 grams of fat-free dry tissue.

When the group of animals developing block is compared with those in which critical signs of potassium poisoning were not seen it is impossible to detect significantly higher concentrations of potassium in the "poisoned" hearts. Although the potassium concentrations expressed per unit weight of intracellular water were also uniformly higher than those found in normal hearts there was no significant difference between the poisoned and non-poisoned group. Even the reduction of intracellular phase volume by the injection of hypertonic sodium chloride-sodium bicarbonate solution failed to reveal any relation between intracellular potassium concentration and heart block.

The influence of potassium on the redistribution of sodium and chloride. Normal cat heart muscle contains sodium in excess of that which is present as a plasma ultrafiltrate. This conclusion is based upon calculation of the extracellular phase volume of the heart from the Cl_t (tissue chloride) and the $[Cl]_e$ (plasma ultrafiltrate or extracellular phase of chloride concentration)

$$(1) \qquad \frac{(Cl)_s \times 1000}{(H_2O)_s \times 0.96} = [Cl]_e$$

where $(Cl)_s$ is the serum chloride concentration and $(H_2O)_s$, the serum water; $[Cl]_e$ equals the chloride concentration in the extracellular phase.

The size of the extracellular phase is derived from:

$$(2) \qquad \frac{(Cl)_t \times 1000}{[Cl]_e} = (H_2O)_e,$$

extracellular water in grams per 100 grams of fat-free dry tissue, where $(Cl)_t$ is tissue chloride in m.eq. per 100 grams of fat-free dry tissue.

The sodium contained in the extracellular phase as a plasma ultrafiltrate is:

$$(3) \qquad \frac{[Na]_e \times (H_2O)_e}{1000} = (Na)_f,$$

$=$ m.eq. of extracellular phase sodium per 100 grams of fat-free dry tissue.

If all of the sodium present is dissolved as a plasma ultrafiltrate, then:

$$(Na)_t = (Na)_f$$

Substitution in equation (3) of values obtained on normal cat hearts gives:

$$\frac{155.8 \times 144}{1000} = 22.4 = (Na)_f$$

$=$ m.eq. of extracellular phase sodium per 100 grams of fat-free dry tissue.

The Na$_t$ obtained by analysis of heart muscle is 24.4 m.eq. per 100 grams of fat-free dry tissue, or 2 m.eq. per 100 grams which are either in the cells or in some way removed from free diffusion through extracellular fluid.

The data from experiments on KCl-treated rats show a relative increase of chloride over sodium. This observation indicates that the volume of extracellular fluid that would contain sodium cannot contain all the chloride. It follows, therefore, that extracellular fluid cannot be measured from the serum chloride and tissue chloride but must be measured by similar equations involving

TABLE 2

Penetration of intracellular space by chloride in potassium-treated cats

CAT	BLOCK	[Na]$_e$	[Cl]$_e$	H$_2$O$_e$ (BASIS Na)	Na$_t$	Cl$_t$	[Cl]$_t$	"EXTRA CHLORIDE" [Cl]$_t$ − [Cl]$_e$
		m.eq. per liter serum ultrafiltrate			m.eq. per 100 gm. fat-free dry tissue			
							m.eq./l.	
2	—	162.0	126.3	149	24.1	19.8	132.8	6.5
4	—	150.3	133.3	142	21.4	22.2	156.3	23.0
7	—	150.5	127.4	121	18.2	18.0	148.8	21.4
9	—	133.0	136.2	149	19.9	21.1	141.6	5.4
11	—	166.0	141.6	147	24.4	24.6	167.3	25.7
13	—	147.2	131.2	134	19.7	18.1	135.1	3.9
Mean $\overline{X}$............								14.3
S.E. $\overline{X}$............								±5.3
1	+	160.6	124.3	140	22.5	20.5	146.4	22.1
3	+	168.9	131.5	164	27.7	23.0	140.2	8.7
5	+	161.5	135.1	139	22.4	21.8	156.8	21.7
6	+	149.0	123.7	130	19.4	22.0	169.2	45.5
8	+	149.1	140.5	120	17.9	19.0	158.3	17.8
10	+	154.0	137.5	171	26.3	25.3	148.0	10.5
12	+	141.0	131.2	155	21.9	25.1	161.9	30.7
14	+	156.0	135.7	136	22.2	23.0	169.1	33.4
Mean $\overline{X}$............								23.8
S.E. $\overline{X}$............								±5.7
P								0.04

sodium. Using these equations one obtains values for tissue chloride in excess of the amount which could be present as a constituent of plasma ultrafiltrate. This "excess chloride" is either within the cells or in some way excluded from free diffusion through the extracellular phase.

Table 2 presents data on animals receiving KCl only. The "excess chloride" in the hearts of the potassium poisoned animals reached a mean of 23.8 ± SE $\overline{X}$ 5.7 m.eq. per liter, while animals showing no signs of poisoning had 14.3 ± SE $\overline{X}$ 5.3 m.eq. per liter. The difference between these two means is *statistically* significant, having a P value of 0.04 obtained by the use of Fisher's formulae and probability tables for small series (14).

The displacement of sodium and the apparent intracellular penetration of chloride shown by the data in table 2 are observations which are consistent with the concepts of Conway and Boyle (12) and of Dean (13) who points out that in skeletal muscle "... when external potassium is high there will be so little sodium in the fibers ... that it will be difficult to establish that the fibers contain sodium."

DISCUSSION. The above experiments show that there is no necessary relationship between the amount of potassium found in the heart and the appearance of signs of potassium poisoning. Heart muscle, like skeletal muscle, in the presence of increasing concentrations of potassium in the plasma takes up potassium and loses sodium from the cells. In addition to these exchanges of monovalent-basic ions in the heart muscle there is evidence that chloride either penetrates the cell membranes or becomes occluded in such a way that part of it is no longer freely dissolved in the extracellular phase. Hearts showing signs of potassium poisoning are found to contain a larger proportion of intracellular chloride than those which were unaffected. If a strict quantitative relation existed between the amount of intracellular chloride and the appearance of signs of poisoning it should be similar to that demonstrated by Winkler, Hoff and Smith (10) for serum potassium and the electrocardiographic evidence of potassium influence on the heart. While a general association of large chloride penetration with poisoning can be shown, it is not possible to detect a uniform trend in this relationship.

The rate at which the potassium rises in the serum and presumably the rate of its penetration of cells in the heart is an important factor. It was observed that in every case but one where signs of poisoning appeared at serum concentrations below 10.0 m.eq. per liter, the rate of rise was above 0.33 m.eq. per liter per minute. This serves to emphasize that the tissue and blood samples were obtained in these experiments at a time when changes were taking place rapidly. Therefore, it is not surprising that the electrolyte and water distribution do not conform to the values which were predicted by the equations based upon equilibrium states in muscle by Conway and Boyle (12), Dean (13) and other workers. The variability of heart water and intracellular electrolyte concentrations observed in our experiments is consistent with similar phenomena observed in cats subjected to alteration of extracellular electrolyte concentration (6).

Since the electrocardiographic evidence of potassium poisoning is characterized by signs of diffuse interference with conduction, it is possible that these changes are the result of damage to which the cells of the conducting system are more susceptible than the rest of the heart. Critical changes in the pattern of distribution of inorganic ions might occur in the Purkinje system and be concealed from detection by chemical analysis of the whole heart. In addition it is known that damaged cells may lose their ability to maintain the physiological distribution of inorganic ions and tend to behave as inert physico-chemical systems at the mercy of diffusion pressures. If widely dispersed groups of heart muscle fibers were damaged by the increasing potassium concentrations or by

events which were the consequence of the rise in potassium, the electrolyte analysis of the whole heart would yield values which would represent the sum of both intact and damaged cell contents in unknown proportions. While our experiments contain no specific evidence on this point, it is possible that the effects we observed were secondary to coronary vasoconstriction induced by the high serum potassium concentrations (15).

SUMMARY

1. Heart, skeletal muscle and serum electrolytes and water analyses were made on 23 cats in which the electrocardiograph was used to detect potassium poisoning.

2. Potassium poisoning, as judged from the disappearance of P waves from the electrocardiogram, occurred when the serum potassium reached 11.0 m.eq. per liter, but was detected in some cases at lower serum concentrations if the rate of rise of potassium was faster than 0.33 m.eq. per liter per minute.

3. No quantitative relationship could be demonstrated between the amount of potassium found in the heart and the appearance of potassium poisoning, although both the heart and skeletal muscle were found to take up potassium readily if the plasma concentration is elevated.

4. The uptake of potassium by the heart is associated with the loss of sodium and the appearance of considerable quantities of chloride in the intracellular phase.

5. The above observations are discussed in relation to their significance in potassium poisoning.

REFERENCES

(1) HARRISON, H. E. AND D. C. DARROW.   J. Clin. Investigation **17**: 77, 1938.
(2) STEINBACH, H. B.   J. Cell. and Comp. Physiol. **15**: 1, 1940.
(3) DEAN, R. B.   Proc. Soc. Exper. Biol. and Med. **45**: 817, 1940.
(4) STEINBACH, H. B.   J. Biol. Chem. **133**: 695, 1940.
(5) STEINBACH, H. B.   Cold Spring Harbor Symposia on Quantitative Biology **8**: 242, 1940.
(6) YANNET, H. AND D C. DARROW.   J. Biol. Chem. **134**: 721, 1940.
(7) FENN, W. O.   Physiol. Rev. **16**: 450, 1936.
(8) MILLER, H. C. AND D. C. DARROW.   This Journal **129**: 264, 1940.
(9) MILLER, H. C. AND D. C. DARROW.   This Journal **130**: 747, 1940.
(10) WINKLER, A. W., H. E. HOFF AND P. K. SMITH.   This Journal **124**: 478, 1938.
(11) WINKLER, A. W. AND P. K. SMITH.   J. Biol. Chem. **124**: 589, 1938.
(12) CONWAY, E. J. AND P. J. BOYLE.   Nature **144**: 709, 1939.
(13) DEAN, R. B.   Biological Symposia **3**: 331, 1941.
(14) FISHER, R. A.   Statistical methods for research workers.   4th ed., Oliver and Boyd, Edinburgh, 1933.
(15) KATZ, L. N. AND E. LINDNER.   This Journal **124**: 155, 1938.

RESPONSES OF THE HEART TO REFLEX ACTIVATION OF THE RIGHT AND LEFT VAGUS NERVES BY THE PRESSOR COMPOUNDS, NEOSYNEPHRIN AND PITRESSIN[1]

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A difference in the distribution of the right as compared with the left vagus nerve supply of the heart is known from the results of electrical stimulation of the cut nerves. Thus stimulation of the right vagus commonly results in a sinus bradycardia or sino-atrial block while similar excitation of the left vagus often produces atrio-ventricular dissociation, i.e., A-V heart block, in addition to depression of the S-A node (3, 4, 9, 20). Anatomical studies (17, 22) have not yielded clear-cut evidence regarding a difference in the distribution of the cardiac vagi. In general they agree that the parasympathetic preganglionic fibers terminate in ganglia above the coronary sulcus, that no such terminations are to be found in the ventricles and that the latter receive few if any postganglionic parasympathetic fibers.

Reflex excitation of the vagus nerves in response to massage of the carotid sinuses of man (18, 21), and to a rise of blood pressure produced by pressor compounds such as pitressin (5, 6, 7, 19) and ephedrine (2, 14) often results in A-V block as well as depression of the sino-atrial node. The present report represents an attempt to analyze the rôle of each vagus in the production of the changes in the heart beat which occur in response to a rise of blood pressure.

METHOD. Healthy full-grown dogs were trained to lie quietly while attached in the usual manner to the leads of an electrocardiograph and while submitting to venepuncture. In the majority of preparations the sympathetic innervation of the heart had been abolished by bilateral removal of the stellate and upper 6 thoracic sympathetic ganglia. In several the medullae of the adrenal glands had been destroyed by cauterization. Vagotomy was performed high in the neck. In those cases in which both vagi were cut the animals were kept in good condition for several days by the administration of 10 per cent glucose by vein. Four groups of dogs were prepared. In the first group consisting of 14 dogs the right vagus was cut and the left vagus remained intact. Of this group 9 had been subjected to bilateral removal of the sympathetic nerves of the heart, 2 to both removal of the sympathetics of the heart and destruction of the adrenal medullae, 1 to removal of the right sympathetics of the heart, and 2 to no previous operations.

In the second group consisting of 18 dogs the left vagus was cut and the right vagus remained intact. Of those in this group 6 had been subjected to bilateral

removal of the sympathetic nerve supply of the heart and to destruction of the adrenal medullae, 6 to bilateral removal of the sympathetic nerve supply of the heart, and 6 to no previous operations.

The third group consisted of 20 dogs in which both vagi remained intact. Four of these had been subjected to removal of the sympathetic nerve supply of the heart and destruction of the adrenal medullae, 6 to adrenal demedullation, 3 to removal of the sympathetic nerve supply of the heart, and 7 to no previous operation.

The fourth group consisted of 28 dogs in which both vagus nerves had been cut. Eleven of these animals previously had been subjected to bilateral removal of the sympathetic nerve supply of the heart and to destruction of the adrenal medullae, 12 to removal of the sympathetic nerve supply, and 5 to no previous operations.

Electrocardiograms were recorded continuously during a period beginning 15 to 30 seconds before onset of the injection of the pressor compounds neosynephrin hydrochloride, pitressin,[3] or angiotonin,[4] and ending after the period of maximal depression of the heart. The neosynephrin was injected intravenously by means of a constant-rate injection apparatus at the rate of 1, 2 or 4 cc. of a 1 to 10,000 dilution per minute. Injections were continued usually during a period of 60 to 90 seconds. Pitressin was injected intravenously in a dose of 0.5 to 1.0 pressor unit, or in a few experiments intramuscularly or subcutaneously in a dose of 1.2 to 4.0 pressor units. Angiotonin equivalent to a 1:250,000 adrenalin solution was injected at the rate of 4 cc. per minute by means of the constant-rate injection apparatus. A short period of rest on the table after insertion of the needle in a vein permitted the heart to reach a fairly constant rate before onset of injections. All experiments were performed on the unanesthetized animal.

In the interpretation of the records A-V heart block was said to be present when one or more P waves failed to be followed by a ventricular complex.

RESULTS.   1. *Dogs whose right vagus had been cut and whose left vagus remained intact, i.e., left vagus dogs.*   In 27 of a total of 37 experiments on 14 dogs a partial A-V heart block occurred in response to the injection of neosynephrin. Only one animal of the series failed to respond with at least one record of such a block. In all experiments there occurred a marked slowing of the atria, the rate falling from a pre-injection average of 93 beats per minute to a post-injection average minimum of 54 per minute. Ectopic beats of ventricular origin were observed frequently after the first three or four 15-second intervals following the onset of injection.

In 7 of a series of 10 experiments on 7 of the dogs of this group a partial A-V heart block occurred following the injection of pitressin. Only 1 of the 7 animals failed to develop a block at least once. All of the experiments resulted in slowing

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[3] Parke Davis preparation.
[4] Made available in sufficient amounts to perform a few experiments through the courtesy of Dr. K. K. Chen, Eli Lilly and Co., Indianapolis.

of the atria, the rate falling from a pre-injection average rate of 94 beats per minute to a post-injection average minimum of 58 per minute.

In a single experiment the injection of angiotonin was followed by the development of a 2:1 A-V heart block, as well as atrial slowing from a pre-injection level of 60 per minute to a post-injection minimum of 38 per minute.

The results of this group of experiments are included in table 1 and illustrated in figure 1.

2. *Dogs whose left vagus had been cut and whose right vagus remained intact, i.e., right vagus dogs.* In 28 experiments on the 12 dogs of this group there oc-

TABLE 1

The rôle of the right and left vagus nerves in the depression of the heart which occurs in response to the rise of blood pressure produced by neosynephrin, pitressin and angiotonin

TYPE OF ANIMAL*	PRESSOR COMPOUND USED	NO. OF EXPERIMENTS	NO. OF DOGS USED	AVERAGE ATRIAL RATES†			NO. OF EXPERIMENTS SHOWING			
				Before injection	During 120 seconds after injection		A-V nodal block	A-V nodal rhythm	A-V nodal beats	Ectopic vent. beats
					Highest	Lowest				
Left	Neosynephrin	37	14	93	93	54	27	0	0	7
vagus	Pitressin	10	7	94	93	58	7	0	0	0
intact	Angiotonin	1	1	60	60	38	1	0	0	0
Right	Neosynephrin	28	12	89	92	50	0	4	1	9
vagus	Pitressin	12	6	92	85	42	0	1	1	0
intact	Angiotonin	1	1	135	128	90	0	0·	0	0
Both	Neosynephrin	39	13	81	86	52	8	1	0	3
vagi	Pitressin	9	4	92	90	30	2	0	0	0
intact	Angiotonin	1	1	81	81	81	0	0	0	0
Both	Neosynephrin	44	26	115	133	114	0	0	0	8
vagi	Pitressin	12	11	125	125	118	0	0	0	0
cut	Angiotonin	1	1	158	160	153	0	0	0	0

* Left vagus intact refers to the dogs in which the right vagus had been cut and the left remained intact. Right vagus intact refers to those in which the left vagus had been cut and the right remained intact.

† Average atrial rates represent arithmetical average of the rates per minute determined during 15 second intervals.

curred no instances of A-V heart block in response to the administration of neosynephrin. Intense vagal depression of the heart however did occur as manifested by the slowing of the heart rate from a pre-injection average of 89 per minute to a post-injection average minimum of 50 per minute. In 4 of the 28 experiments, 2 dogs developed A-V nodal rhythm, and in 1 additional case a few A-V nodal beats were recorded. Ectopic ventricular beats were noted in 9 experiments.

In 12 experiments on 6 dogs of this group the administration of pitressin was followed by marked slowing of the atria, the average pre-injection rate being 92 beats per minute and the average post-injection minimal rate being 42 per minute.

There was recorded one instance of A-V nodal rhythm, one of A-V nodal beats and no case of A-V heart block.

In a single trial angiotonin produced only a slowing of the atria from a pre-injection rate of 135 beats per minute to a post-injection minimal rate of 90 per minute.

The results of this group of experiments are included in table 1 and illustrated in figure 1.

3. *Dogs in which both vagus nerves remained intact.* In only 8 of a total of 39 experiments involving 6 of the 13 dogs studied, administration of neosynephrin was followed by a partial A-V heart block. In one experiment A-V nodal rhythm was recorded. The average pre-injection atrial rate was 81 beats per minute and the average minimal post-injection rate was 52 per minute.

In only 2 experiments on 2 animals of a series of 9 experiments on 4 animals, the injection of pitressin resulted in partial A-V heart block. A-V nodal rhythm and A-V nodal beats were not observed in this group of experiments. The average pre-injection atrial rate was 92 per minute and the average minimal post-injection rate was 30 per minute.

In the single experiment of this group in which it was used, angiotonin failed to produce a change of heart rate.

The results of this group of experiments are included in table 1 and illustrated in figure 1.

4. *Dogs in which both vagus nerves had been cut.* In 44 experiments on 26 vagotomized dogs no A-V heart blocks, A-V nodal rhythms or A-V nodal beats developed in response to the administration of neosynephrin. The average pre-injection atrial rate was 115 per minute, the average minimal post-injection rate was 114 per minute, and the average post-injection maximal rate was 133 per minute.

In 12 experiments on 11 of the vagotomized dogs, pitressin produced no A-V heart block, no A-V nodal rhythm and no A-V nodal beats. The average pre-injection rate in these 12 experiments was 125 beats per minute, the average maximal post-injection rate was 125 per minute, and the average minimal post-injection rate was 118 per minute.

The results of this group of experiments are included in table 1 and illustrated in figure 1.

In order to demonstrate the relative importance of direct cardiac effects of neosynephrin data were studied from a series of 41 experiments on 23 of the dogs whose hearts had been completely denervated by bilateral removal of the stellate and upper 5 or 6 thoracic sympathetic ganglia and bilateral vagotomy high in the neck. The results of these experiments are illustrated in table 2. They are concerned with heart rates counted before and during 15 second intervals after the onset of injection of neosynephrin hydrochloride. During the first 15 to 30 seconds after the onset of injection, slowing of the heart of more than 2 beats per minute was recorded in only 2 of the 41 experiments. In one of these the rate was decreased 4 beats and in the other 12 beats per minute. Subsequent to the first or second 15 second interval after the onset of injection, the heart

rate was increased in all but 2 of the 41 experiments. The increases ranged from 0 to 72 beats per minute, or from 0 to 70 per cent. In 31 of the experiments they ranged from 0 to 22 beats per minute, or from 0 to 21 per cent. The time at which the maximal increase occurred ranged from 15 to 165 seconds after onset of injection. In 34 of the 41 experiments the maximal increase in heart rate occurred between 60 and 90 seconds after the onset of injection. In 2 cases ventricular tachycardia developed after a period of acceleration had been recorded. A premature systolic arrhythmia (pulsus bigeminus) was recorded in one case. A typical experiment of this group is shown in figure 1.

The effect of pitressin on the completely denervated heart is demonstrated by the data of table 3. In 7 of the 9 experiments on 8 dogs, the injection of pitressin was followed either by no depression of heart rate or by a slowing of not more than 6 beats per minute. In 1 case there developed a slowing from

TABLE 2

Effect of neosynephrin on the denervated heart—10 typical experiments

DOG NO.	WEIGHT	ADRENAL MEDULLA	CC. PER MIN.*	HEART BEATS PER MINUTE							
				Before injection	15 sec. periods after inject.						
					1	2	3	4	5	6	7
	kilos										
17	10.5	Intact	2	96	96	96	102	108	116	120	
19	10.5	Intact	2	110	110	110	114	118	126	136	146
30	9.1	Intact	4	110	110	112	120	126	136	148	
14	9.5	Intact	2	126	126	128	132	134	136	136	138
21	13.2	Intact	2	128	128	128	132	134	134	140	
22	8.2	Intact	4	120	120	128	148	162	176	188	192
25	13.6	Out	4	130	130	130	132	136	140	150	
28	10.5	Out	4	118	118	124	132	142	150	158	165
34	9.1	Out	4	92	94	94	100	112	128	160	136
35	13.6	Out	2	98	98	100	100	102	104	106	106

* The neosynephrin was injected as a 1 to 10,000 dilution.

a pre-injection rate of 114 beats per minute to a post-injection rate of 94 per minute and in another from 108 to 100 per minute.

DISCUSSION. The advantages to be gained by avoidance of anesthesia in experiments on the reflex depression of the heart in response to a rise of blood pressure are well-known. They concern principally the fact that commonly used anesthetics depress the vagal reflexes. Similarly there are at least two distinct advantages of reflex stimulation as compared with direct electrical stimulation of the fibers of the vagus nerve. The first of these concerns the fact that electrical stimulation may well produce effects which are in excess of those which occur physiologically. The second concerns the possible presence of cardio-accelerator fibers in the vagus nerve (10). It may be assumed that any such fibers are activated along with the inhibitory fibers when electrical stimuli are applied. On the other hand the response of the fibers of the vagus to the physiological stimulus incident to a rise of blood pressure may well concern not
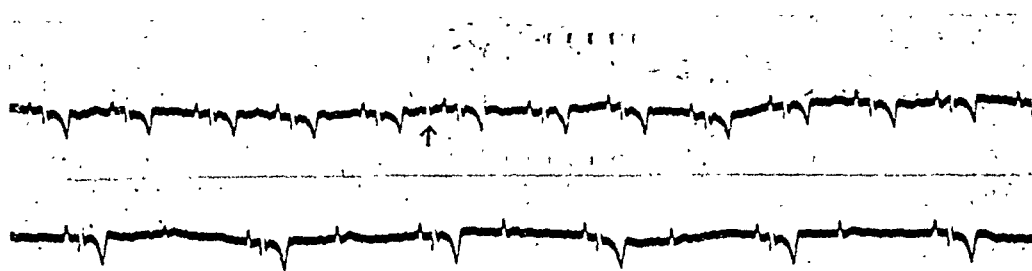
Dog 5 - Left vagus dog. Upper segment: Before injection and onset of injection, at artefact marked by arrow, of neosynephrin 1-10,000 at rate of 4cc per minute.. Lower segment: 2:1 A·V block 49 seconds after onset of injection
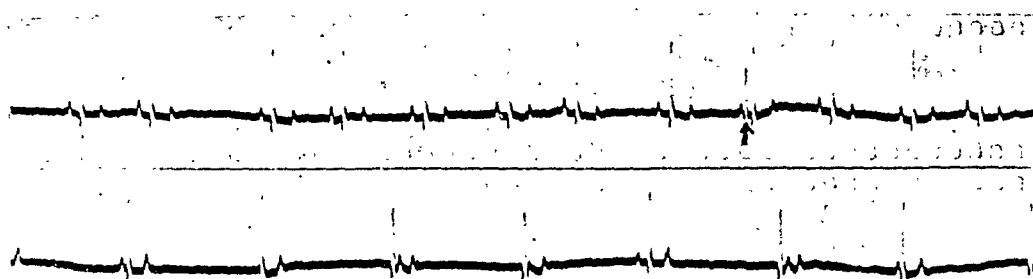
Dog 4. Right vagus dog. Upper segment: Before injection and onset of injection, at artefact marked by arrow, of neosynephrin 1-10,000 at rate of 2c.c. per minute. Lower segment: A·V rhythm 90 seconds after onset of injection
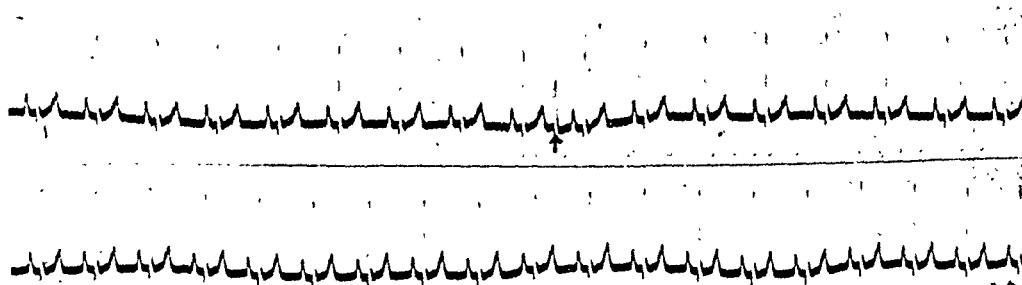
Dog 2. Denervated Heart Upper segment: Before injection and onset of injection, at artefact marked by arrow, of neosynephrin 1-10,000 at rate of 1c.c. per minute. Rate 113/min. Lower segment: Moderate tachycardia, 60 seconds after onset of inj. Rate 128/min.
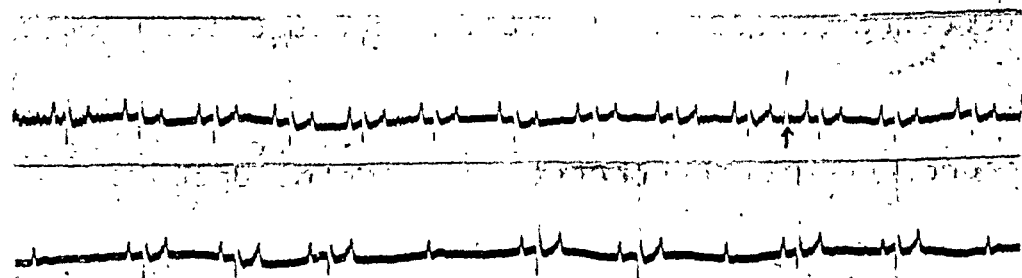
Dog 26. Left vagus dog. Upper record: Before injection and onset of injection, at artefact marked by arrow, of Pitressin 1 ¼ pressor units. Lower record: Partial A·V. block first evident at 22 seconds after onset of injection

Fig. 1

only activation of the vagal cardio-inhibitory fibers but also reciprocal inhibition of cardio-accelerator fibers. It seems clear then that there are very distinct advantages to be gained by the use of the method outlined.

During the early experiments on the problem the animals were subjected to removal of the sympathetic nerve supply of the heart and destructive cauterization of the adrenal medullae before carrying out the experiments on reflex activation of the vagi. It soon became apparent however that these procedures had no obvious influence on the results insofar as they are concerned with the production of heart block. In the later experiments as indicated under method most experiments were carried out on animals whose sympathetic nerves to the heart and whose adrenal medullae remained intact. It of course must be recognized that the heart rates in the case of the latter animals may be influenced to some extent by reflex depression of the sympatho-adrenal mechanism (1). The heart rates presented in table 1 must be interpreted with the above facts in mind. They are included in the table in order to illustrate the degree to which the

TABLE 3

Effect of pitressin on the denervated heart

DOG NO.	WEIGHT	ADRENAL MEDULLA	DOSE	HEART BEATS PER MINUTE								
				Before injection	15 sec. periods after injection							
					1	2	3	4	5	6	7	8
	kilos		*p.u.**									
22	8.2	Intact	1.6*	88	88	88	88	88	88	88	88	
25	13.6	Out	0.5	114	114	108	102	98	96	98	96	94
30	9.1	Intact	2.0*	108	106	106	106	106	106	106	106	
34	9.1	Out	0.5	108	106	102	104	104	104	100	102	
34	9.1	Out	1.0	90	90	88	88	92	90	94	96	90
35	13.6	Out	0.5	96	96	95	96	96	102	102		
55	12.3	Out	1.0	144	144	144	142	142	144			
57	17.0	Out	1.0	130	130	130	130	126	124			
58	9.1	Out	1.0	162	162	160	160	162				

* Dose is recorded in pressor units. In the case of dog 22 the injection was intramuscular and in that of dog 30 subcutaneous. All others were intravenous.

heart was affected in response to the pressor compound injected. Thus it is clear that the heart rate was profoundly depressed both by neosynephrin and by pitressin. Similarly it is evident that the rate is depressed to about the same degree in the left vagus group as it is in the right vagus group. In view of these facts the difference in the distribution of A-V heart block appears to be significant.

The pressor compounds neosynephrin, pitressin and angiotonin were selected because of the fact that each produces a rise of blood pressure in response to peripheral vasoconstriction. The direct stimulatory influence of neosynephrin on the heart has been partially avoided by taking advantage of the fact that the threshold for direct cardiac stimulation by the compound is relatively high as compared with that for vasoconstrictor action (23). However as noted in figure 1 neosynephrin produced some elevation of the rate of the denervated heart in those experiments in which the lowest of the 3 injection rates was employed. As indicated in the results and in table 2 neosynephrin failed to

depress the rate of the denervated heart in all but 2 of 41 experiments on 23 of the dogs. The two exceptions represented in one case a decrease of 4 beats per minute and in the other of 12 beats per minute. Both occurred during the first 15 second interval following the onset of injection. Following this interval the rate increased in all but two of the 41 experiments.

These results do not support the suggestion of Keys and Violante (11) that neosynephrin is capable of producing a primary bradycardia by inhibition of the sino-atrial node, and that this inhibition is independent of blood pressure reflexes involving the vagus nerve. They would seem to offer strong evidence against a direct inhibitory influence of neosynephrin on the heart. Therefore the recorded evidences of cardiac depression in the normal dogs and in those having partially denervated hearts used in the present study are interpreted on the basis of reflexes which arise as a result of the increase of blood pressure produced by the vasoconstriction caused by neosynephrin.

The dosage of neosynephrin was varied as indicated in the results. The rate of 1 cc. per minute was used in 2 of the experiments on right vagus dogs, in 2 on left vagus dogs, in 2 on those in which both vagi had been cut, and in none on those in which both vagi remained intact. In the remaining experiments the choice between a rate of 2 cc. and 4 cc. per minute was made usually on the basis of size of the animal. Examination of the data reveals no evidence that variation of injection rate played any part in controlling the incidence of A-V block or A-V nodal rhythm as recorded in table 1.

The pressor compound pitressin undoubtedly produces depression of the heart principally through reflexes initiated as a result of the rise of blood pressure which follows its vaso-constrictor action. However evidence has been presented by Kolls and Geiling (13), Resnik and Geiling (19), Geiling and Resnik (5), and Gruber and Kountz (6, 7) that removal of vagal action by atropinization or by vagotomy does not abolish although it does reduce the cardiac depressant action of pitressin. This action was attributed by Gruber and Kountz (6, 7) to coronary vasoconstriction and direct effects of the compound on the myocardium. Melville (16) reported that ephedrine prevents the depressant action of posterior pituitary extract on the vagotomized or atropinized heart. He attributed this result to the vasodilator influence of ephedrine on the coronary vessels and concluded that posterior pituitary depresses the heart in part through its vasoconstrictor action on these blood vessels. It may reasonably be assumed that reflex depression of the sympathetic cardio-accelerator mechanism was responsible for at least a part of the slowing observed in the experiments of the above authors.

As indicated by the data of table 3 and results published in a previous report (8) pitressin either fails to produce slowing or causes only a moderate slowing of the completely denervated heart. Thus of the 9 experiments on 8 dogs recorded in table 3, 6 show no reduction of more than 2 beats per minute and the remaining 3 a reduction of 6, 8 and 20 beats. Of the 3 experiments included in the previous report (8) 2 indicated no slowing and 1 a slowing of only 2 beats per minute. It seems reasonable on the basis of these results to attribute the depression of

the heart observed in normal dogs and in those having partially denervated hearts principally to reflex influences initiated by the rise of blood pressure produced by the pitressin.

In a single experiment the injection of angiotonin resulted in a decrease in rate of the denervated heart from 158 to 153 beats per minute, a degree of inhibition which may be regarded as insignificant. Therefore the slowing of the heart observed in the normal dogs and in the dogs having partially denervated hearts may be interpreted as being due to reflexes initiated in response to the rise of blood pressure produced by the angiotonin.

The possibility of course must be recognized that any or all of the above 3 pressor agents may exert a direct influence on the activity of the vagal nuclei and thus cause showing of the heart. Such an action has been suggested by Keys and Violante (12) for neosynephrin. In view of the similar responses obtained in the case of the three distinct pressor compounds, it has not seemed necessary to postulate that a direct central action of neosynephrin was involved in our experiments.

The results obtained on the left vagus dogs, i.e., those whose right vagus was cut and left vagus remained intact, indicate that depressed A-V nodal conduction is a prominent feature of the inhibition produced reflexly through the left vagus. Thus a partial A-V nodal block was recorded following the injection of neosynephrin in 27 of a total of 37 experiments on 14 dogs of this group. A similar result was observed in 7 of 10 experiments on 7 of the left vagus dogs following injection of pitressin, and in a single experiment in which angiotonin was used. It will be noted that marked depression of the S-A node is a common feature of the experiments involving depression of the heart through the left vagus. It is clear then that reflex excitation of the left vagus in response to a rise of blood pressure in the unanesthetized animal is capable of resulting in depression of the S-A node to the point of producing marked sinus bradycardia, and the A-V node to the point of producing A-V heart block.

In the 28 experiments on 12 dogs prepared by removal of the left vagus, i.e., right vagus dogs, sinus bradycardia uniformly was the most prominent feature of the response to the pressor compounds. In no case was there evidence of any degree of A-V heart block. An A-V nodal rhythm was observed following the administration of neosynephrin in 4 experiments involving 2 dogs and a few A-V nodal beats were recorded in a single additional case. Similar results were recorded in experiments involving pitressin in which all of the 12 experiments on 6 dogs demonstrated a marked sinus bradycardia, 1 an A-V nodal rhythm, and none an A-V block. A single experiment using angiotonin resulted only in a sinus bradycardia. These results offer no evidence in favor of a depressor action of the right vagus nerve on the A-V node. Examination of the P-R intervals of the electrocardiograms of this series reveals no evidence of depression of A-V conduction.

As indicated in table 1, A-V nodal rhythm was recorded in a total of 5 experiments on right vagus dogs. This result is in agreement with that of Meek and Eyster (15) who observed A-V rhythm in 2 of 27 experiments in which the right

vagus of the dog was stimulated electrically. The development of this rhythm may be explained by assuming that the automaticity of the S-A node was depressed to a level below that of the A-V node by impulses traveling in the right vagus. In those experiments on right vagus dogs in which neosynephrin was used, A-V rhythm may have been due in part to a direct stimulatory action of this compound on the A-V node. Such an effect would occur at a time when the S-A node was being subjected to the depressant action of the right vagus. That a stimulatory action on the A-V node is not essential to the development of A-V rhythm is indicated by the fact that pitressin resulted in the production of this rhythm in 1 right vagus dog.

SUMMARY

The pressor compounds neosynephrin, pitressin and angiotonin are capable of producing an inhibition of the heart which is best explained on the basis of reflexes initiated in response to the rise of blood pressure resulting from their vasoconstrictor action. Administration of these pressor compounds may be said to result in a physiological activation of the vagus nerves.

In experiments on dogs whose right vagus has been cut and whose left vagus remains intact, i.e., left vagus dogs, the response to the pressor compounds commonly includes A-V heart block. Thus in this group of animals A-V block followed the injection of neosynephrin in 27 of 37 experiments on 14 dogs, of pitressin in 7 of 10 experiments on 7 dogs and of angiotonin in a single experiment.

In dogs whose left vagus had been cut and whose right vagus remained intact, i.e., right vagus dogs, no instances of A-V heart block occurred in response to injection of neosynephrin in 28 experiments on 12 dogs, of pitressin in 12 experiments on 6 dogs and of angiotonin in a single experiment.

Physiological activation of either the right or the left vagus results in sinus bradycardia. No remarkable difference in the degree of heart slowing produced by the left as compared with the right vagus is evident.

As a result of physiological activation of the right vagus the automaticity of the S-A node frequently is depressed to a level at which the A-V node takes over the function of pacemaker. Thus A-V nodal rhythm was observed in 4 of 28 experiments using neosynephrin and in 1 of 12 experiments using pitressin. A-V nodal rhythm occurred in none of the experiments involving the left vagus group of dogs.

The completely denervated heart of the dog is not depressed by neosynephrin in the doses in which it was employed.

In the majority of experiments pitressin fails to cause significant inhibition of the completely denervated heart. When inhibition occurs it usually is of small degree.

REFERENCES

(1) ALLEN, W. F.   This Journal 110: 602, 1935.
(2) CHEN, K. K. AND W. J. MEEK.   J. Pharmacol. and Exper. Therap. 28: 31, 1926.
(3) COHN, A. E.   J. Exper. Med. 18: 715, 1913.
(4) COPE, O. M. AND H. C. COOMBS.   Proc. Soc. Exper. Biol. and Med. 33: 480, 1935.

(5) GEILING, E. M. K. AND W. H. RESNIK.   J. Clin. Investigation 1: 239, 1925.

(6) GRUBER, C. M. AND W. B. KOUNTZ.   J. Pharmacol. and Exper. Therap. **39**: 275, 1930.

(7) GRUBER, C. M. AND W. B. KOUNTZ.   J. Pharmacol. and Exper. Therap. **40**: 253, 1930.

(8) HANEY, H. F. AND A. J. LINDGREN.   J. Pharmacol. and Exper. Therap. **76**: 363, 1942.

(9) HERING, H. E.   Pflüger's Arch. **108**: 281, 1905.

(10) KABAT, H.   This Journal **128**: 246, 1940.

(11) KEYS, A. AND A. VIOLANTE.   Proc. Soc. Exper. Biol. and Med. **44**: 4, 1940.

(12) KEYS, A. AND A. VIOLANTE.   J. Clin. Investigation **21**: 1, 1942.

(13) KOLLS, A. C. AND E. M. K. GEILING.   J. Pharmacol. and Exper. Therap. **24**: 67, 1924.

(14) MEEK, W. J. AND M. H. SEEVERS.   J. Pharmacol. and Exper. Therap. **51**: 287, 1934.

(15) MEEK, W. J. AND J. A. E. EYSTER.   Heart **5**: 227, 1914.

(16) MELVILLE, K. I.   J. Pharmacol. and Exper. Therap. **64**: 86, 1938.

(17) NONIDEZ, J. F.   Am. J. Anat. **65**: 361, 1939.

(18) PURKS, W. K.   Ann. Int. Med. **13**: 270, 1939.

(19) RESNIK, W. H. AND E. M. K. GEILING.   J. Clin. Investigation 1: 217, 1925.

(20) ROTHBERGER, C. J.   Ergebn. d. Physiol. **32**: 598, 1931.

(21) SIGLER, L. H.   Am. Heart. J. **9**: 782, 1934.

(22) WOOLLARD, H. H.   J. Anat. **60**: 345, 1926.

(23) YOUMANS, W. B., H. F. HANEY AND K. W. AUMANN.   This Journal **130**: 190, 1940.

POTASSIUM AND THE CAUSE OF DEATH IN TRAUMATIC SHOCK[1,2,3]

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Increase in the concentration of potassium in serum has been repeatedly reported in various types of secondary shock (1, 11). It has been suggested that this elevated concentration of potassium may be an important factor in producing the characteristic picture of traumatic shock, and may contribute to the fatal outcome. On the other hand, the picture of potassium poisoning is totally unlike that of traumatic shock (2, 3, 4). Also, concentrations of potassium considerably higher than those commonly found in shock are necessary in order to elicit any clinical evidences of toxicity (2, 5). The present study endeavors to define as exactly as possible the rôle of potassium in one variety of traumatic shock.

METHODS. Thirty-five dogs were used. Initial intraperitoneal dial anesthesia was employed. Shock was produced by release of a tourniquet constricting one or more limbs, the tourniquet having been applied some hours before with sufficient firmness to occlude the arterial supply completely (6). Within an hour after the release of the constriction the affected limb had swollen markedly below the level of constriction, while there was an associated decline in the plasma volume to about 60 per cent of normal (7). Blood pressure was low, the peripheral pulse was weak and unobtainable, and venous return from the periphery was markedly reduced. This state of affairs continued for a period varying from one to thirty-six hours, when death took place. At intervals blood samples were obtained for serum potassium analysis (8). In some experiments nonprotein nitrogen content of whole blood was determined (9). Electrocardiograms were obtained before and at frequent intervals after the establishment of shock. Every effort was made to observe the exact sequence of events at the moment of death, and to obtain electrocardiograms and blood samples just before and just after this event. Unfortunately this was not always possible. In certain supplementary experiments the effects of acute fatal anoxia on the serum potassium and on the electrocardiogram of the normal dog were studied.

Electrocardiographic criteria of potassium poisoning. Potassium in elevated concentrations characteristically affects the electrocardiogram (2, 4, 10). Concentrations above normal but less than 8 or 9 mM per liter affect the height and character of the T waves, and depress the ST segment. At a level of 9 or 10 mM per liter P waves disappear. Slightly higher concentrations cause a widen-

ing of the QRS complexes (intraventricular block), and a slight further increase in concentration is followed by cardiac arrest in diastole. Until intraventricular block is quite extreme, these changes are reversible with falling concentration, and are not accompanied by any change in blood pressure or respiration. During the extreme slowing and advanced intraventricular block just preceding complete arrest dyspnea first appears. Death characteristically occurs with cardiac arrest while the respirations are still very active. Since this sequence of events is invariably present with rising concentration of potassium, is found in no other known condition, and produces a mode of death which is so characteristic, there
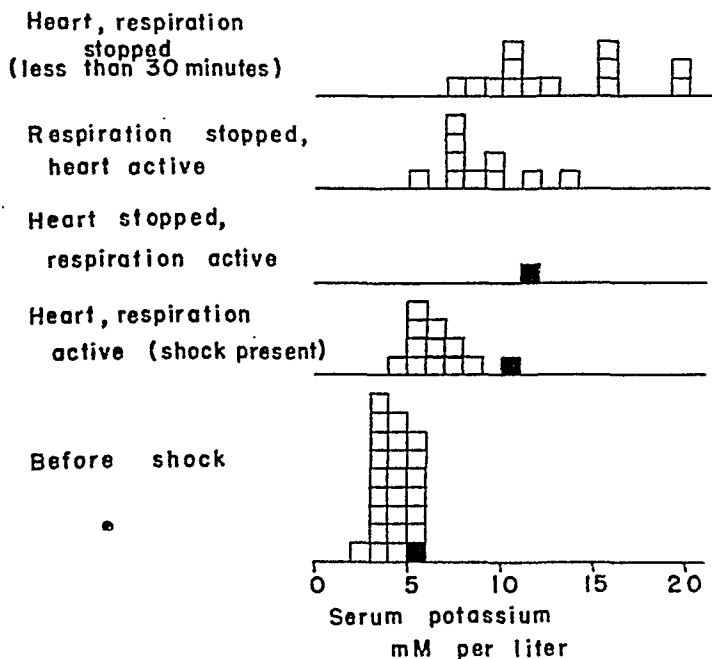
Fig. 1. Concentrations of potassium in serum at various stages of secondary shock. Each square represents a single chemical determination. The concentration of potassium in serum was usually determined more than once in each animal. The solid squares represent successive determinations in the single animal which certainly died of potassium poisoning.

should be no difficulty in determining whether or not potassium poisoning played any significant rôle in these experiments.

RESULTS. A. *Serum potassium concentration and mode of death.* In figure 1 the serum potassium concentrations from all experiments are related to the circumstances attending death in traumatic shock. This classification was necessary in view of the rapid changes which may occur in serum potassium concentration immediately following respiratory failure. Frequently there was an abrupt rise with cessation of breathing, while the heart was still beating. There was also very commonly a further sharp rise in the serum potassium within fifteen to thirty minutes after both heart and respirations had stopped, even before intravascular clotting had taken place. It is accordingly of the utmost importance in each experiment to determine the exact relationship of the serum sample taken for analysis to cardiac and respiratory activity. In the lowest tier

is shown the distribution of initial concentrations, while just above are represented the concentrations found at all stages of shock preceding death itself. On a statistical basis there is clearly an increased concentration of serum potassium after shock is well established. It is also true that there is a rise in each individual experiment. In only one instance, however, did the concentration exceed 9 mM per liter (solid square). This occurred in the same animal whose serum potassium concentrations are represented by the solid squares above. In this one experiment the heart stopped while respirations were still active, a mode of death found in potassium poisoning, the serum potassium concentration both before and at the moment of cardiac arrest exceeding 10 mM per liter. The electrocardiographic changes were also indicative of potassium poisoning. This was the exceptional event; much more commonly respiration stopped while the heart was still actively beating. The concentrations of potassium in sera obtained after respirations had ceased but while the heart was still beating are shown in the line next to the top. Although six of the values are less than 8 mM per liter, two lie between 8 and 10, while two exceed 10 mM per liter. In the top line are shown the concentrations of potassium in uncoagulated sera obtained by cardiac puncture from two to twenty-five minutes after cessation of both respirations and heart. They illustrate the rapidity with which serum potassium may increase postmortem.

B. *Changes in blood nonprotein nitrogen concentration.* The concentration of nonprotein nitrogen in whole blood was usually somewhat elevated at the time of death. In eighteen experiments in which this determination was made at or near the time of death, the average value was 69 mgm. per cent. There was a rough correlation with the time of survival following the development of shock. The highest value, 143 mgm. per cent, was found in the one certain case of death from spontaneous potassium poisoning, and the next highest, 113 mgm. per cent, in one of the suspected cases. Both these animals survived an exceptionally long time. All other values were distinctly lower, often not much exceeding 40 mgm. per cent. Correlation with terminal concentration of potassium was poor, mainly because of the rapid variation in the latter. Only in the case with the maximal elevation was the increase in the non-protein nitrogen concentration of the same general magnitude as that seen in animals with complete anuria (4). More often the rate of increase was rather less rapid. It is possible that some, but not all, of the increase of serum potassium in shock results from failure of normal renal excretion.

C. *Electrocardiographic changes.* (1) *Changes while respiration is active.* During this period electrocardiographic changes were confined to inversion or change in the height of the T waves, with a lowering of the ST segment,[4] in all but three experiments. P waves were unaffected, changes in QRS complexes and block of any sort were notably absent. In the three exceptional cases changes of another sort gradually developed. Following certain T wave changes,

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[4] *Figures presenting actual electrocardiograms illustrative of the various changes described in the ensuing paragraphs have been omitted in the interests of economy of publication.*

the P waves disappeared, and intraventricular block then developed. Cardiac arrest occurred while respirations were still active. A short period of dyspnea followed cardiac arrest. This is the same unique case represented by the solid squares in figure 1, whose serum potassium was greater than 10 mM per liter *before* any alteration of respiration or of cardiac automaticity. The electrocardiographic sequence, the mode of death, and the level of potassium in serum both before and at the time of death, are all entirely characteristic of potassium poisoning.

(2) *Changes after respirations ceased or became ineffective.* These were found in all except the three exceptional experiments noted in the preceding paragraph. In a few cases cardiac and respiratory arrest were almost simultaneous, but in most instances the electrocardiographic complexes persisted for some time after respiratory arrest. Occasionally there would be one final gasp after a minute or so of complete apnea, and this might coincide with the final complexes. As was pointed out in the preceding paragraph, the changes prior to respiratory arrest were confined to alterations in the T wave and in the ST segment. After respiratory arrest at least two sequences were observed. Complexes of the usual form might continue until cardiac arrest, usually with a few disorganized complexes just before the end. The serum potassium in these cases never exceeded 8 mM per liter. On the other hand, a sequence of changes usually associated with a rising concentration of serum potassium might make their appearance in the few moments *following* respiratory arrest. In a typical instance of this kind the serum potassium at the end of the sequence was 12.2 mM per liter, although not long before respiratory arrest it had been only 7 mM per liter. The reasons for the marked rise of serum potassium in some experiments but not in others is obscure. It seems reasonable to interpret the second type of sequence as a secondary reaction of the agonal electrocardiogram to the terminal release of potassium. The true cause of death, however, is the respiratory failure with consequent anoxemia.

D. *Effects of acute fatal anoxia on serum potassium.* In figure 2 are presented the effects of sudden respiratory cessation on the serum potassium of dogs not in a state of shock. After dial anesthesia was established, the chest was opened and artificial respiration instituted. After a time this was suddenly stopped. An elevation of the serum potassium developed within a minute or two, while the heart was still beating actively. These experiments are frequently complicated by decline in the serum potassium during the period of artificial respiration, whether as a result of overventilation or from some other cause. The experiments nevertheless prove that an acute terminal rise in the serum potassium with respiratory failure may occur in animals whose circulatory status was normal, as well as in those suffering from shock. In the latter state the serum potassium prior to respiratory arrest was usually higher, so that the concentrations attained in the blood at the time of final arrest were also frequently higher than those found in the experiments of figure 2.

DISCUSSION. These experiments confirm the regular appearance of an increased serum potassium in traumatic shock. General tissue anoxia and defec-

tive renal excretion both contribute to this increase. However, this increase of serum potassium is as a rule too small to cause any serious cardiac derangement. The electrocardiographic evidence alone suffices to prove that cardiac arrest due to potassium poisoning is not the usual cause of death in this type of traumatic shock. Indeed, the absence of any premortal electrocardiographic changes ex-
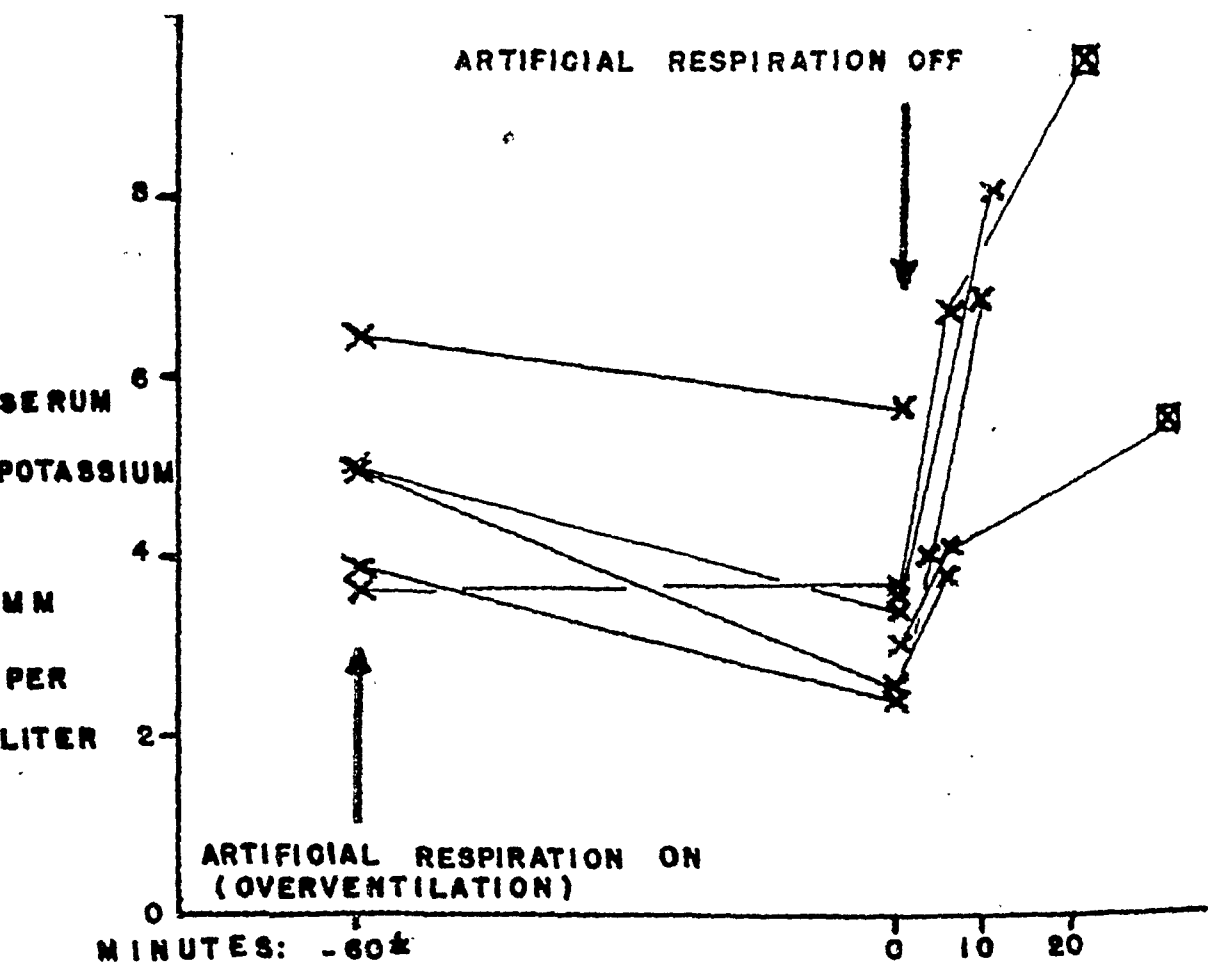
Fig. 2. The effects of sudden acute fatal anoxia on the serum potassium of dogs not in shock. The experimental procedure is described in the text. Crosses indicate determinations made while the heart was still beating, squares samples obtained after cardiac arrest. There is regularly an acute rise in the concentration of potassium in serum within five to ten minutes after stopping artificial respiration.

cept T wave inversion emphasizes the probable relative unimportance of myocardial damage in the functional pathology of shock.

At the same time these studies do explain some inconsistencies in the literature. It is true that typical cardiac arrest from potassium autointoxication may exceptionally be the terminal event. The single completely proven case occurred in an animal remaining in a state of shock for an unusually long period. This animal's renal function must have been almost abolished during the period of shock, since the blood nonprotein nitrogen after thirty-six hours of shock had

risen to 143 mgm. per cent. This increase is as great or greater than that observed in completely anuric animals in the same period of time (4). The serum potassium increased more than is usual in simple anuria of this duration. It may be supposed that more of the animals would have died in this fashion, had not a more speedy form of death, due to quite different causes, overtaken them before sufficient potassium could accumulate. Attempts to produce potassium poisoning as a terminal event by the use of less extensive initial ischemia of the leg resulted only in recovery of the animals. Evidently fatal potassium poisoning as the terminal event depends on shock of sufficient severity to cause renal failure and eventual death of the animal, yet not severe enough to produce death more acutely from respiratory failure.

These experiments also indicate the probable interpretation of the high terminal concentrations of serum potassium which have sometimes been reported (1, 11). Unless special care was taken to obtain the specimens from the still active heart, they may have been truly postmortem samples. Since potassium content of cardiac blood rises swiftly as soon as the heart stops beating, an artificially high concentration of serum potassium might have resulted. In many instances, however, unusually high potassium concentrations have been reported in samples of blood obtained while the heart was still beating. Our observations and those of others (12) indicate that there is a regular premortal, terminal rise in the potassium concentration of serum from cardiac blood (fig. 2), and that at times this increase may reach a considerable height. However, sharply increased concentrations of serum potassium and associated electrocardiographic changes do not appear until *after* respiration has ceased. They are therefore the *effect*, not the *cause*, of the agonal sequence of events. It is true that these terminal increases in concentration of potassium may themselves be responsible for certain of the final stages in the electrocardiographic sequence, and that these changes may therefore simulate those of spontaneous potassium poisoning. However, their appearance only after respiratory failure gives the clue to their real significance.

CONCLUSIONS

1. Concentration of potassium in serum consistently increases when secondary shock is induced by re-establishment of circulation in an ischemic limb.

2. Death usually occurs with respiratory failure prior to or simultaneous with cardiac arrest, and is usually not due to potassium poisoning. Concentrations of potassium in serum while both respiration and heart are active in the shocked animal seldom exceed 8 mM per liter. These levels are too low to cause any circulatory embarrassment; minor electrocardiographic changes only are noted.

3. Exceptionally, death may be due to cardiac arrest from spontaneous autointoxication with potassium. Long persistence of the shocked state with failure of renal excretion favors this event. It is characterized by the attainment of concentrations of potassium in serum exceeding 10 mM per liter while both heart and respiration are active, and by cardiac arrest while the respirations are still normally active.

4. After respiratory arrest, there may be a rapid agonal elevation of the serum potassium. Such increases are the result and not the cause of the terminal event.

REFERENCES

(1) SCUDDER, J. Shock. Blood studies as a guide to therapy. J. B. Lippincott Co., 1940.
(2) WINKLER, A. W., H. E. HOFF AND P. K. SMITH. This Journal 124: 478, 1938.
(3) HOFF, H. E., P. K. SMITH AND A. W. WINKLER. This Journal 127: 722, 1939.
(4) HOFF, H. E., P. K. SMITH AND A. W. WINKLER. J. Clin. Investigation 20: 607, 1941.
(5) SCUDDER, J., M. E. SMITH AND C. R. DREW. This Journal 126: 337, 1939.
(6) SWINGLE, W. W., J. W. REMINGTON, W. KLEINBERG, V. A. DRILL AND W. J. EVERSOLE. This Journal 138: 156, 1942.
(7) HOPPER, J. AND A. W. WINKLER. Unpublished observations.
(8) HALD, P. M. J. Biol. Chem. 103: 471, 1933.
(9) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Volume II. Methods. Williams & Wilkins, 1931.
(10) WINKLER, A. W., H. E. HOFF AND P. K. SMITH. J. Clin. Investigation 20: 119, 1941.
(11) MANERY, J. F. AND D. Y. SOLANDT: This Journal 138: 499, 1943.
(12) DENNIS, J. AND R. M. MOORE. This Journal 123: 443, 1938.

EFFECTS PRODUCED BY VITAMIN D ON ENERGY, APPETITE, AND OESTROUS CYCLES OF RATS KEPT ON AN EXCLUSIVE DIET OF YELLOW CORN[1]

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In previous experiments (1) young rats were kept on a diet in which yellow corn constituted the sole source of nourishment. For the first 20–30 days on this diet the rats showed only minor effects. After that they became progressively less active, lost their appetite, lost their 4–5 day oestrous cycles showing only constant dioestrous smears, and failed to gain weight. After the rats had shown these deficiency symptoms constantly for over two months, cod liver oil was made accessible to them in a separate container, with the result that within only a few days' time they lost most of the evidences of deficiency. Their intake of corn increased, they started to become active again, gained weight, and within 4 days showed regular 4 day oestrous cycles. After 15 days rats which had been running less than 1 mile per day, ran over 18 miles per day. The rats appeared to be in fine health, had smooth coats, good teeth, kept themselves clean, and showed no signs of deficiency. Thus, under the conditions of these experiments, yellow corn supplemented with cod liver oil became a complete food.

The multiple components of cod liver oil made it impossible to conclude whether one or several of the substances, vitamin A or D, or one or more of the fatty acids, was responsible for the marked improvement in nutrition. Further experiments have now been made in which some of these substances were offered separately in purified form, to rats which for a long period of time had been kept on an exclusive diet of yellow corn.

METHODS. The technique and the conditions were duplicated from the previous experiments. The rats were placed in the activity cages and given our stock diet[2] at an average age of 45 days and were started on the exclusive yellow corn diet fifteen days later. The yellow hybrid corn (no. g-94)[3] was finely ground (4225 mesh per sq. in.).

Records were made daily of food and water intake, running activity (number of revolutions of running drum), and vaginal smears; weekly records were made of body weight and of the general appearance of the animals as to coat, eyes, teeth, tail, paws, etc. Thirteen piebald female rats were used. After 100 days on the corn diet the first group of 4 rats were given access to a solution of vitamin D_3 in specially prepared vitamin- and sterol-free cotton seed oil (50 U.S.P.

[2] Graham flour 725; crude casein (Adler) 100; skim milk powder (Breadlac) 100; butter 50; calcium carbonate 15; sodium chloride 10.

[3] The corn for these experiments was kindly furnished by Funk Bros. Seed Co., Bloomington, Ill.

units/gram) for 40 days; the 3 rats in the second group were subjected to ultra-violet radiation, using a quartz mercury-vapor lamp,[4] after 130 days on the corn diet; after 100 days on the diet, the 3 rats in the third group were offered vitamin A acetate dissolved in the same cotton seed oil (500 I.U./gram); and the 3 rats in the fourth group were offered a vitamin A- and D-free distillate of cod liver oil (20 per cent distillate in cotton seed oil).[5]
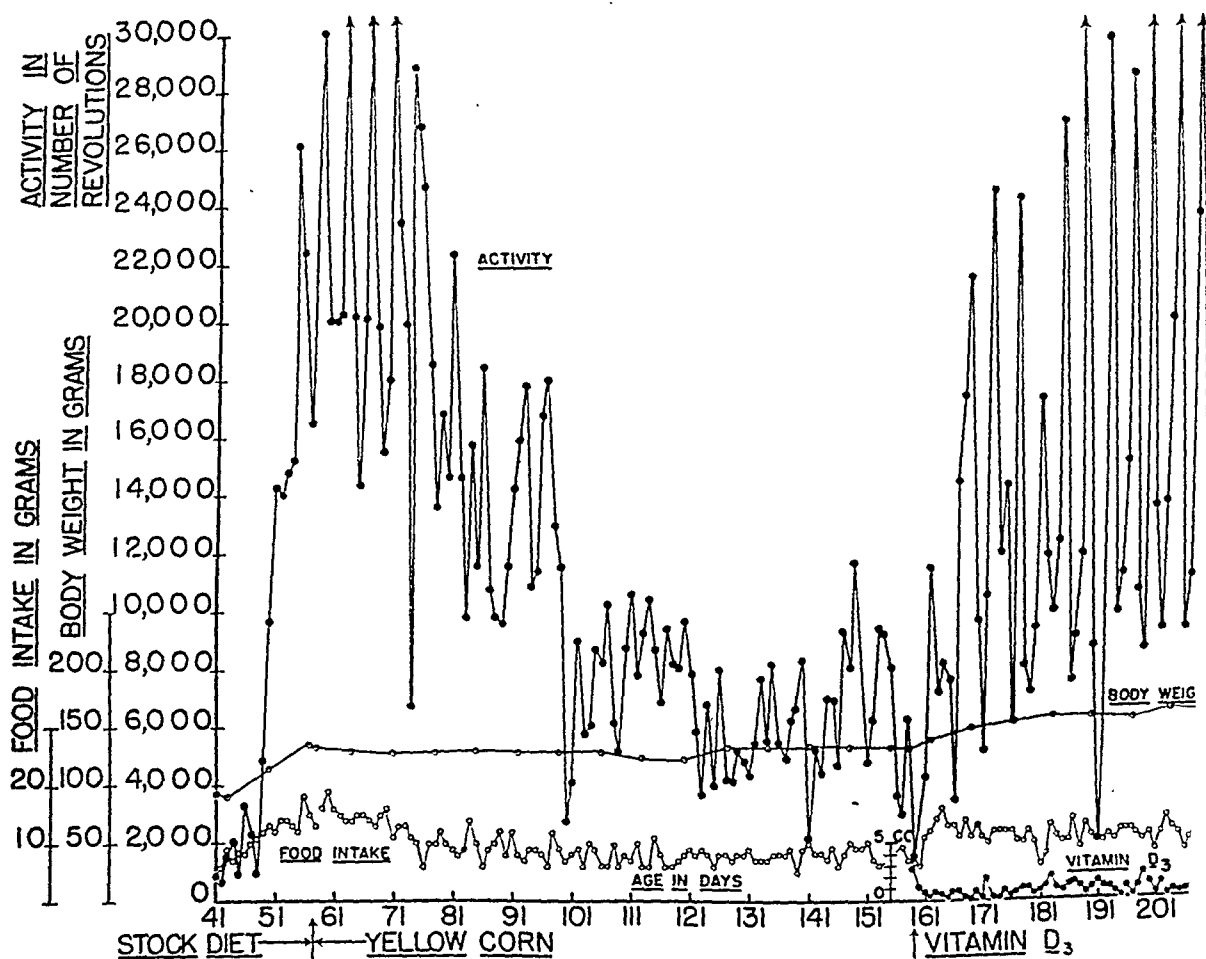
Fig. 1. Showing the activity, food intake, and body weight of a rat maintained on an exclusive diet of yellow corn and tap water for 100 days, and the effect of making a solution of vitamin D_3 available.

RESULTS. Figure 1 gives a typical record of an individual animal kept on an exclusive diet of yellow corn for 100 days, followed by 50 days with access to a solution of vitamin D_3 in cotton seed oil, showing daily activity, food and oil intake, and body weight. The activity (shown in revolutions of the drum) averaged nearly 16,000 revolutions per day for the last 10 days on stock food, in-

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[4] The lamp was kindly loaned by the Dept. of Physiotherapy of the Johns Hopkins Hospital. It was the electrodeless high-frequency type, manufactured by Lepel Laboratories, Inc., N. Y.

[5] These preparations were provided through the courtesy of Distillation Products, Inc., Rochester, New York.

creased to more than 20,000 revolutions during the first 20 days on the corn diet, and then fell within 30 days to a level near 7,000, which was maintained with little variation for the remaining 50 days on the corn diet. During the first 30 days, when the activity remained high, it showed very regular 4–5 day cycles, with the peaks of activity accurately coinciding with the days of cornification of the vaginal smears; later, as the activity decreased and the vaginal smears revealed a constant state of dioestrus, the cyclical variation was lost. When vitamin D_3 was offered, the activity began to increase within 10 days, reaching 16,000 revolutions per day during the next 40 days. Concurrently with the increase in activity (and the return of normal vaginal cycles), the 4–5 day peaks of activity were again evident.

The food intake rose from a daily average of 13.6 grams for the last 10 days on the stock diet to 15.1 grams for the first 10 days on the corn diet. Ninety days later it had fallen nearly 50 per cent, to 8.6 grams. The addition of vitmin D_3 caused an immediate increase in appetite, the food consumption averaging 11.7 grams for the first 10 days with the supplement. During the 100 days on the corn diet the body weight of the animal was almost exactly maintained, varying between 126 and 134 grams. The addition of vitamin D_3 caused an increase in weight to 141 grams in the first 10 days, and a further increase to 159 grams during the 31–40th day period. The vaginal smears, which with the exception of the few periods of cornification, had shown only the epithelial cells and leucocytes characteristic of a dioestrous condition, changed within 8 days to regular 4 day oestrous cycles.

Effects on spontaneous activity. Table 1 summarizes the results of the changes produced on the activity of the four groups of rats. The average daily activity of the 4 rats of the vitamin D_3 group decreased from 18,980 revolutions for the first 10 days on corn to 3,707 for the 91–100th day on corn and then increased to 12,820 for the 31–40th day period after the rats had access to vitamin D_3. All 4 rats showed essentially the same effect.

The activity of the 3 rats in the second group decreased from an average of 15,553 revolutions per day during the first 10 days on the diet to 3,845 revolutions during the 91–100th day period. The ultraviolet radiation was not begun until the animals had been on the diet 130 days, at which time the activity had decreased still further, to an average of 1,378 revolutions per day. The rats were exposed at a distance of 26 inches and the time of exposure increased by one minute daily, from 5 to 22 minutes, without noticeable difference in effect; consequently after the 19th day the exposure was maintained for 15 minutes daily at a distance of 26 inches. The average daily activity had increased to 2,701 revolutions during the 31–40th day period of radiation, with 2 of the 3 rats showing an increase. On the 54th day of irradiation the animals were given access to a solution of pure cholesterol[6] and the irradiation continued as before. During the period from the 8–18th days with the cholesterol the activity increased

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[6] The cholesterol was also furnished by Distillation Products, Inc., and was dissolved in the same cotton seed oil used as vehicle for the other preparations, at a concentration of 31 mgm. of cholesterol per cc.

further, to an average of 3157 revolutions, though only one animal showed a pronounced effect.

The activity of the 3 rats of the vitamin A acetate group decreased from an average of 20,518 revolutions for the first 10 days on corn to 3,864 for

TABLE 1

RAT NO.	AVERAGE DAILY ACTIVITY—NUMBER OF REVOLUTIONS OF DRUM			AVERAGE DAILY FOOD INTAKE IN GRAMS			AVERAGE BODY WEIGHT IN GRAMS		
	First 10 days on corn diet	Last 10 days on corn diet	31–40th days with supplement	First 10 days on corn diet	Last 10 days on corn diet	31–40th days with supplement	First 10 days on corn diet	Last 10 days on corn diet	31–40th days with supplement
Group I—yellow corn + vitamin D₃									
364y	23,932	6,996	15,962	15.1	8.6	10.8	131	133	159
365y	20,150	2,006	14,628	14.8	7.6	11.8	132	134	148
367y	14,636	2,559	9,900	13.2	6.1	11.3	136	120	146
369y	17,205	3,266	10,790	13.0	5.6	9.9	126	110	121
Average.....	18,980	3,707	12,820	14.0	7.0	11.0	131	124	144
Group II—yellow corn + ultra violet irradiation									
338z	6,385	721	1,382	13.0	10.2	9.3	145	173	164
355z	16,166	1,614	1,018	13.3	7.7	9.3	139	134	152
357z	24,108	1,800	5,703	12.9	7.1	11.8	134	142	180
Average.....	15,553	1,378	2,701	13.1	8.3	10.1	139	150	165
Group III—yellow corn + vitamin A acetate									
363y	21,300	1,117	582	12.9	5.2	6.5	117	107	103
366y	18,891	4,408	2,985	14.7	6.9	7.6	127	133	130
5z	21,364	6,068	9,193	13.2	8.3	7.4	150	151	143
Average.....	20,518	3,864	4,253	13.6	6.8	7.2	131	130	125
Group IV—yellow corn + cod liver oil distillate									
368y	19,195	1,869	654	14.6	6.1	7.4	135	123	120
6z	19,547	4,052	2,681	13.8	7.2	7.5	147	140	144
10z	25,972	5,066	13	13.6	6.5	7.5	135	123	94
Average.....	21,571	3,662	1,116	14.0	6.6	7.5	139	129	119

the 91–100th day period on the corn diet and increased only slightly, to 4,253, for the 31–40th day on vitamin A acetate. With the vitamin, the activity of one animal increased slightly, while that of the others continued to decrease.

The activity of the 3 rats of the cod liver oil distillate group decreased from a daily average of 21,571 revolutions for the first 10 days on corn to 3,662 for the 91–100th day period and decreased further to 1,116 for the 31–40th day after

access was given to cod liver oil distillate.   All 3 rats became less active on the concentrate.

Effects on appetite.   Table 1 shows that the average daily food intake of group I decreased from 14.0 grams for the first 10 days on corn to 7.0 grams for the 91–100th day period, i.e., 50 per cent, and increased to 11.0 grams for the 31–40th day period on the vitamin D_3 supplement.   However, although the food intake increased sharply on the supplement, it did not attain its originally high level. All 4 rats showed essentially the same change.

The second group also showed a decrease in food intake while on the corn diet, from an average of 13.1 grams during the first 10 days to 9.8 grams in the 91–100th day period, and a still further decrease to 8.3 grams during the 131–140th days, immediately before the start of ultraviolet radiation.   During the 31–40th days of treatment the average food consumption was 10.1 grams, which was almost unaffected by the subsequent addition of cholesterol.

The average food intake of the rats in group III decreased from 13.6 to 6.8 grams on corn and increased slightly, to 7.2 grams, on the vitamin A acetate. Two rats showed an increase, and one a decrease with the supplement.

The food intake of the group IV rats decreased from an average of 14.0 to 6.6 grams on corn and increased slightly, to 7.5 grams, on the cod liver oil distillate.

Effects on body weight.   Table 1 also shows the changes produced on body weight.   The body weights of the rats in all 4 groups remained approximately the same throughout the time on the corn diet.   However, when they were given access to the different vitamin preparations, marked differences were brought out.   The animals given vitamin D_3 all began to gain weight promptly, increasing from an average weight of 124 to 144 grams in 40 days, and those irradiated with the quartz mercury vapor lamp also gained weight, from 150 to 165 grams, though less rapidly.   On the other hand, the rats in the other 2 groups not only failed to gain, but actually lost weight: those given vitamin A decreased from 130 to 125 grams, while those given the vitamin-free distillate of cod liver oil decreased from 129 to 119 grams, on the average.

Effects on oestrous cycles.   During the period on the stock diet all 13 rats showed fairly regular 4 day cycles of cornification, which were maintained with the same regularity for from 12 to 50 days on the corn diet.   After this time 6 of the animals remained in a state of constant dioestrus throughout the remaining time on corn, while 6 continued to show rare days of cornification during the next 15 to 75 days, and one exceptional animal at no time showed any prolonged abnormality of the oestrous cycle.   When the various supplements were added, the effects on the 4 groups were again widely different.   Whereas 3 of the 4 rats in the vitamin D_3 group showed regular cycles starting within 8 days, and continuing throughout the 40 days of the experimental period, and all 3 irradiated animals also showed regular cycles in 1, 6, and 26 days respectively, none of the 6 rats in the other 2 groups showed any change from their previous state of constant dioestrus.

DISCUSSION.   The present results show that in the previous experiments the

effects produced by cod liver oil on the energy, appetite, and sex cycles of rats kept on a diet of yellow corn exclusively, must have resulted in large part from the vitamin D content of the cod liver oil.   The results showed, further, that when supplemented with vitamin D_3, yellow corn became almost a complete food, and that, to a lesser extent, ultraviolet irradiation of the animals had a similar beneficial effect.   However, if the experiments had been continued over longer periods of time, or if the rats had been mated, some deficiencies might have manifested themselves.   Definite proof has thus been obtained of the dependence of energy output, appetite, and the condition of the reproductive tract on vitamin D, under these conditions.

The observation that rats show less energy on a vitamin D deficient diet is in agreement with the reports of several other workers.   Seel (2) found that the basal metabolic rate was lowered in rickets and could be restored to normal by vitamin D treatment.   Many other workers have confirmed this observation. Reed et al. (3) and Reed (4) reported that vitamin D treatment increased the metabolic rate in dogs and in rats.   Ziegler and Knudson (5) and Nicolaysen (6) also found that rats kept on a rachitic diet became less active and showed a lowered food intake.

The marked depression in activity produced by the lack of vitamin D and the sharp increase in activity produced by the vitamin D treatment, and to a lesser extent by ultraviolet irradiation, might suggest either a primary effect on the thyroid gland or a direct effect on carbohydrate metabolism.   It is conceivable that without vitamin D the rats could not utilize the large amount of carbohydrate present in corn.

The probability must also be considered that changes in calcium metabolism played an important part in this phenomenon.   Corn is known to have an extremely low calcium content, so low that on theoretical grounds it is surprising that rats should be able to exist so long on a diet limited to corn.   The vitamin D presumably brought about a more efficient utilization of the small amounts of calcium that are present in corn.

Several workers had previously reported either direct or indirect effects of vitamin D on the reproductive tract.   Frey (7) found that vitamin D in the form of vigantol affected the sex cycles of rats and Agduhr (8) reported that moderately large doses of ergosterol increased the rate of reproduction of rabbits. In human beings clinical observations, made usually in connection with vitamin D treatment of other conditions, have shown that large doses of vitamin D may have a definite effect on the reproductive tract.   Thus Reed, Struck and Steck reported that in 12 of 388 women experimentally given massive vitamin D therapy for arthritis and miscellaneous other conditions there was a definite estrogenic effect (9).   In the most marked case a 23 year old woman, with a history of irregular menses since puberty at the age of 12, with inter-menstrual periods varying from 16 days to 3 months, menstruated every 28 days during the 4 months of therapy, and gradually regressed to her previous irregularity within 6 months of the cessation of the vitamin D administration.   In the light of our experimental results, it seems possible that the 12 women showing the estro-

genic response were either actually deficient in or showed a heightened requirement for vitamin D.

The close similarity between the symptoms and the appearances of the vitamin D-deficient single food corn rats and those of hypophysectomized rats—the low level of activity, poor appetite, failure to grow, constant dioestrous condition—would suggest that the deficiency may have a primary effect on the anterior lobe of the pituitary gland. The results of treatment with anterior lobe extracts, and a histological study of the hypophyses of single food corn rats should help to determine the rôle played by the pituitary. If such experiments brought out a close interrelationship, the effect of vitamin D on activity, calcium metabolism, and the reproductive cycles could be interpreted as the result of stimulation of the thyroid, parathyroids, and ovaries by the corresponding anterior pituitary hormones.

SUMMARY

1. The decreased activity, poor food intake, and constant state of dioestrus produced in rats by an exclusive diet of yellow corn, are all corrected by giving the animals access to a solution of pure vitamin D_3.

2. Ultraviolet irradiation of yellow corn fed animals has a similar corrective effect, but to a less marked extent than does the administration of the vitamin.

3. Both vitamin A acetate and a vitamin A- and D-free distillate of cod liver oil were essentially without effect on the nutritive condition of rats maintained on the yellow corn diet for 100 days.

4. Therefore, it is concluded that the supplementary value of cod liver oil on an exclusive diet of yellow corn is, in all probability, due to the vitamin D content of the fish oil.

5. Attention was called to the close similarity between the symptoms of single food corn rats and those of hypophysectomized rats, and the possibility was considered that vitamin D might achieve its effect indirectly through stimulation of the anterior lobe of the hypophysis.

REFERENCES

(1) RICHTER, C. P. AND K. K. RICE. This Journal 139: 147, 1943.
(2) SEEL, H. Arch. f. exper. Path. u. Pharmakol. 140: 194, 1929.
(3) REED, C. I., E. A. THACKER, L. M. DILLMAN AND J. W. WELCH. J. Nutrition 6: 355, 1933.
(4) REED, C. I. Proc. Soc. Exper. Biol. and Med. 32: 274, 1934.
(5) ZIEGLER, L. H. AND A. KNUDSON. J. Comp. Psychol. 22: 199, 1936.
(6) NICOLAYSEN, R. Skandinav. Arch. f. Physiol. 82: 79, 1939.
(7) FREY, E. München. med. Wochnschr. 81: 1009, 1937.
(8) AGDUHR, E. Ztschr. f. Vitaminforsch. 4: 54, 1935.
(9) REED, C. I., H. C. STRUCK AND I. E. STECK. Vitamin D. Univ. of Chicago Press, Chicago, Illinois, 1939, pp. 156–159.

EFFECT OF DIETARY CHANGES UPON URINE VOLUME AND RENAL FUNCTION IN EXPERIMENTAL DIABETES INSIPIDUS

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Considerable attention has been directed upon the relative inability of the kidney to concentrate sodium chloride in the urine in diabetes insipidus (1–7). Indeed, the very existence of the polyuria has been ascribed to the salt contained in the ordinary diet, and the decline in polyuria during fasting has been thought to be due to a lessened excretion of salt (4–6), but this has been shown to be not true for the cat with experimental diabetes insipidus (d.i.) (7). Little attention has been paid in the literature on d.i. to the excretion of substances other than salt, but as a result of the experiments herein reported, it is evident that the d.i. kidney also suffers from relative inability to concentrate nitrogen in the urine. Since, on an ordinary diet, nitrogenous substances form the major portion of the total solids in the urine, deficient ability to concentrate nitrogen is of greater relative importance in the maintenance of the polyuria than is the disability to concentrate salt.

METHODS. Cats and dogs have been used in these experiments. D.i. was produced in the cats either by hypothalamic lesions with a Horsley-Clarke stereotaxic instrument, or by section of the hypophyseal stalk, and in the dogs by stalk section only. The stock diet of the cats consisted of raw ground beef, 80 grams, and milk, 100 cc. daily; for the dogs, 200 grams to 300 grams of commercial dog biscuits. Experimental diets were devised by which we could vary independently the nitrogen, sodium chloride (determined as chloride), or caloric content. Total nitrogen in each diet was determined and corrected by a digestibility factor (9); chloride was estimated by Volhard-Harvey titration after open Carius digestion. Caloric content was not determined, but calculated from tables (10). Urinary nitrogen was determined by a microkjeldahl procedure, with Nesslerization and reading in a photoelectric colorimeter. Creatinine clearances were obtained on trained dogs, about two hours after priming by stomach tube with about 0.2 gram of creatinine per kilogram of body weight. For creatinine analysis, color was developed by the Jaffe reaction and read in the photoelectric colorimeter. In the dog, creatinine clearance may be taken as a measure of glomerular filtration; the figures recorded are averages of at least three clearance periods of approximately twenty minutes each. The U/P ratio (concentration of creatinine in the urine divided by that in the plasma) was determined, and used as an index of tubular reabsorption, for it can be shown that $\dfrac{100\ (U/P - 1)}{U/P}$ = per cent of filtered water reabsorbed by the renal tubules.

OBSERVATIONS. *Effect on urine volume of cats of varying the nitrogen intake.* The routine diet of the cats contained about 3.2 grams of total nitrogen in a

daily portion. On such a regime, a normal cat excretes urine with a total nitrogen concentration averaging about 25 mgm. per cc., but the d.i. cat's urine has only 4 to 10 mgm. nitrogen per cc., depending on the degree of the polyuria. The degree to which a d.i. cat concentrates nitrogen in the urine is fairly constant for any one individual animal under a rather wide range of nitrogen intake, whereas in the normal animal the concentration varies widely under different dietary conditions (table 1).

TABLE 1

Effect of dietary changes on the polyuria of experimental diabetes insipidus in cats
Values shown are averages for 24-hour periods

KIND OF CAT	DIET	NUMBER OF DAYS	INTAKE			URINARY OUTPUT				
			Calories	Cl as NaCl	Total N	Cl as NaCl		Total nitrogen		Volume
				grams	*grams*	*mgm. per cent*	*grams*	*mgm. per cent*	*grams*	*cc.*
d.i.	A, stock	5	228	0.238	2.88	40	0.282	383	2.59	675
	B, high calorie	3	251	0.281	2.73	31	0.217	422	2.85	675
	C, low calorie	3	154	0.193	2.79	38	0.262	401	2.71	672
	D, low nitrogen	4	245	0.110	1.25	31	0.132	403	1.59	395
	E, low calorie low nitrogen + urea	4	148	0.203	2.74	42	0.233	488	2.73	554
	F, high nitrogen	2	237	0.376	4.09	33	0.294	386	3.40	875
	G, low nitrogen	5	236	0.202	0.77	56	0.142	405	1.03	256
d.i.	A, stock	5	228	0.238	2.88	57	0.244	604	2.57	427
	B, high calorie	3	251	0.281	2.73	46	0.218	556	2.77	466
	C, low calorie	3	154	0.193	2.79	49	0.249	558	2.84	507
	D, low nitrogen	3	245	0.110	1.25	63	0.160	579	1.55	267
	E, low calorie low nitrogen + urea	3	148	0.203	2.74	52	0.211	703	2.87	413
	F, high nitrogen	2	237	0.376	4.09	56	0.310	707	3.13	553
	G, low nitrogen	5	141	0.124	0.46	48	0.113	540	1.23	232
Normal	A, stock	14	228	0.238	2.88			2789	2.84	103
	D, low nitrogen	3	245	0.110	1.25			4365	1.10	23
	F, high nitrogen	3	237	0.376	4.09			4288	4.62	102

Figure 1 shows the prompt subsidence of the polyuria which occurs in a d.i. cat when the protein intake is reduced without significant change in caloric or chloride intake. The picture here is identical with that seen during fasting. Experiments similar to the one shown were performed on three other d.i. cats, with similar results. Such experiments suggest that the marked decrease in urine volume of d.i. observed during fasting may be attributed to lessened demand for water for the excretion of urinary nitrogenous compounds, and the reason the d.i. animal exhibits a polyuria under ordinary circumstances is that the kidney is so deficient in ability to concentrate nitrogenous substances that

an amount of nitrogen which can be excreted in a small volume of urine by the normal individual has a strongly diuretic effect in d.i. This interpretation is strengthened by further experiments reported below.

We do not mean to imply that relative inability to concentrate nitrogen is the only deficiency exhibited by the d.i. kidney, for it has been amply demonstrated that a similar dysfunction exists with respect to sodium chloride (1, 2, 3, 7, 8), and it is probable that the same may be found to be true for other urinary constituents as well. But the amount of sodium chloride contained in the stock diet is well within the limit which the d.i. kidney can handle with little or no polyuria (7), whereas the nitrogen ordinarily ingested is in excess of that which
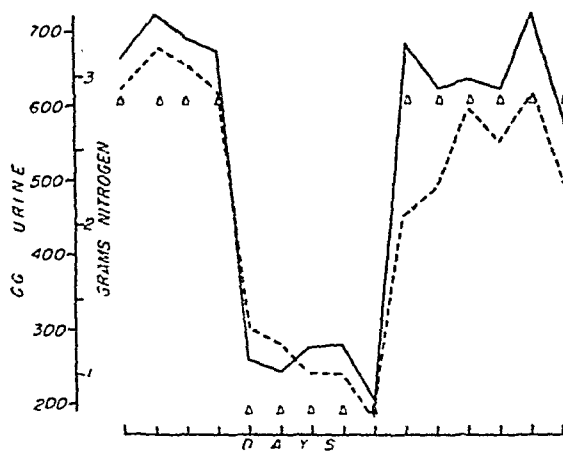
Fig. 1
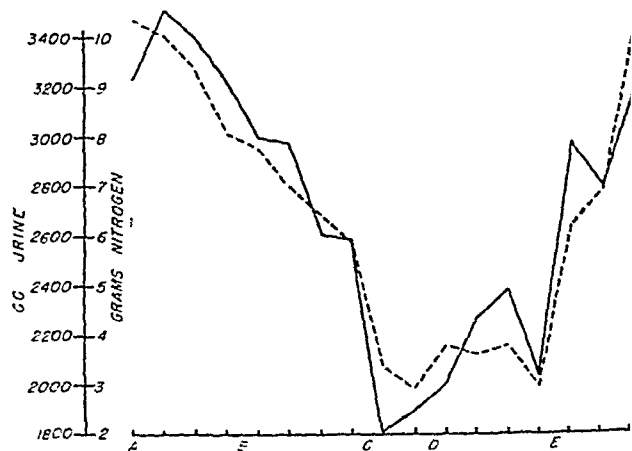
Fig. 2

Fig. 1. Cat with diabetes insipidus. Effect on the polyuria of an abrupt reduction of the protein in the diet, and subsequent return to stock diet. Solid line, urine volume. Broken line, total urinary nitrogen excretion. Triangles, nitrogen intake.

Fig. 2. Dog with diabetes insipidus. Parallelism between nitrogen excretion and urine volume on various diets. Solid line, urine volume. Broken line, total urinary nitrogen excretion. Abscissae, 24-hour periods. Diets as follows: A, stock; B, reduced nitrogen; C, fast; D, low nitrogen; E, stock.

the d.i. animal can excrete in a normal urine volume, and hence there is a polyuria on a normal diet.

The exact degree to which the polyuria could be reduced by a procedure such as is shown in figure 1 varied with different animals, depending on the degree of the initial polyuria. A cat with a very severe d.i., such as is shown in the figure, could not be reduced to a completely normal urine volume (100 to 150 cc.). We did not find it possible to reduce the urinary total nitrogen excretion in cats to a level much below 1 gram a day, either during fasting or with low nitrogen intake. Thus, under these circumstances, the nitrogen output was still sufficient to keep the urine volume somewhat above the level characteristic of a normal animal, except in the case of one d.i. cat that had a relatively mild polyuria even on the stock diet.

Table 1 shows results obtained in a series of experiments designed to determine the effect of different dietary procedures on the urine volume. The experiments

were performed on 5 d.i. and 2 normal cats, and some of them were repeated several times on each cat; the table, therefore, does not show all the results, but those shown are typical. It is to be noted that chloride concentrations are low in all the diets, so that none of the changes in urine volume shown can be attributed to the slight variations in chloride; for example, in a d.i. cat, in order to obtain by sodium chloride alone an increase in urine volume as great as that obtained by high nitrogen (diet F), the chloride content of the diet would have to be 5 to 10 times as great as that actually present (7).

The variations in chloride content of the various diets in table 1 are, therefore, to be regarded as unimportant, because there is not enough chloride in any of them to require a large urine volume for its excretion, even in a d.i. cat. Inspection of the table reveals that the urine volume of the d.i. cats is independent of the caloric intake, at least within the limits employed, but parallels very closely the nitrogen excretion. Diversity in caloric and nitrogen intake was obtained by feeding beef, milk, eggs and suet in varying proportions. Diet E demonstrates that it is indeed the nitrogen and not some other constituent of the diets that is responsible for the results obtained. This was a "low-calorie, low nitrogen" diet, to which enough crystalline urea was added to bring the nitrogen content up to approximately the normal level.

In contrast to the results obtained in d.i. cats, the normal cats responded to a high nitrogen intake by merely increasing the concentration of nitrogen in the urine, without increasing the urine volume. In this respect, the normal cats differed from the dogs (see below), and resembled the rats reported by Gamble *et al.* (11). On a low nitrogen diet, the urine volume of the normal cats dropped as markedly as that of the d.i., but nevertheless the response of the two groups was different. The low nitrogen diet used was not only lower in nitrogen than the stock diet, but lower in water content as well. Since the normal cats drank no water during the time under observation (water was given *ad libitum* during these experiments), they excreted a small volume of highly concentrated urine.

Effect of water restriction. In the experiments reported up to this point, the cats were allowed free access to water. The question arises: if water is withheld from the d.i. cat, can he be forced to concentrate nitrogen in a manner similar to the normal cat? Such "dehydration" experiments are shown in table 2. During the dehydration period, the d.i. cats received their usual diet of meat and milk, but water was completely withheld, except for the animal with the most severe polyuria, which received a total of 200 cc. of water to drink during the first two days, and no water for the rest of the period. Previous experience had shown that such a procedure was sufficient to bring about serious dehydration in d.i. cats. Normal cats can not be dehydrated by merely withholding water, so these animals were not only denied both water and milk, but in addition about one-third of the water in the meat was removed before it was fed. They suffered no ill effects from such treatment; indeed, we had found by previous tests that normal cats could stand such a regimen apparently indefinitely. Thus, it can not be said that the normal animals were actually dehydrated. The experiment does, however, show something of the ability of the normal kidney to concentrate

nitrogen, although the concentrations shown here are not necessarily the maximum of which the animals are capable. The d.i. cats, on the other hand, called upon their bodily reserves of water in order to excrete the nitrogen during the dehydration period, and ran a strongly negative water balance (see also (19)). They appear to be concentrating nitrogen to the maximum of their ability, and yet their concentration is much less than that of the normal cats, even when the latter have free access to water. The d.i. cat under such conditions loses water from his tissues so fast that he is dehydrated to a degree dangerous to his health within a few days, and the experiment has to be discontinued.

Experiments on dogs. Certain comparable experiments were performed on 4 d.i. and 3 normal dogs. Figure 2 shows that in the dog, as in the cat, reduction

TABLE 2

Effect of restricted water intake on urine volume and nitrogen concentration

CONTROL PERIOD					WATER RESTRICTION PERIOD					
Ave. daily urine volume	Ave. daily water drunk	Ave. urine specific gravity	Ave. urinary total nitrogen concentration	Ave. daily total nitrogen in urine	Number of days	Ave. daily urine volume	Ave. daily water drunk	Ave. urine specific gravity	Ave. urinary total nitrogen concentration	Ave. daily total nitrogen in urine
d.i. cats										
cc.	*cc.*		*mgm. per cent*	*grams*			*cc.*		*mgm. per cent*	*grams*
387	282	1.008	689	2.66	5	171	0	1.015	1348	2.30
331	246	1.010	865	2.86	4	166	0	1.020	1660	2.74
616	513	1.006	438	2.70	4	239	50	1.014	983	2.23
408	321	1.009	724	2.94	5	170	0	1.020	1547	2.61
Normal cats										
99	0	1.031	2288	2.29	5	45	0	1.055	6952	2.75
111	0	1.033	2544	2.31	5	48	0	1.060	6420	3.11

in the nitrogen intake leads to a diminished polyuria, although the urine volume does not fall completely to a level characteristic of the normal animal. For the low nitrogen diet, suet was substituted for part of the dog biscuits. Figure 3 shows that the degree of the polyuria parallels the nitrogen output when the sodium chloride intake is not at an abnormally high level. On the other hand, this experiment confirms previously reported results on cats (7) that when the salt intake is increased to a level considerably above that found in an ordinary diet, the urine volume tends to parallel the chloride output, even on a low nitrogen diet. In other words, the deficient concentrating ability of the d.i. kidney is not specific for either salt or nitrogenous substances; the necessity for the excretion of a large quantity of either substance is sufficient for the maintenance of a high degree of polyuria, and in order to reduce the polyuria of d.i. by dietary means alone it is necessary that both the sodium chloride and the nitrogen intake be maintained at a low level.

The lack of any specific effect for either sodium chloride or nitrogenous compounds is further shown by the data presented in figure 4. In these experiments, both chloride and total nitrogen output were determined; the chloride was calculated as os-millimols[1] of NaCl, and the nitrogen as os-millimols of urea. The sum of these two, representing approximately the total osmolar daily excretion of these substances, is plotted against the daily urine volume. It is to be noted that in both the normal and the d.i. dog, the urine volume varies directly with the sum of the osmotic activities of the urea and NaCl excreted. It is recognized
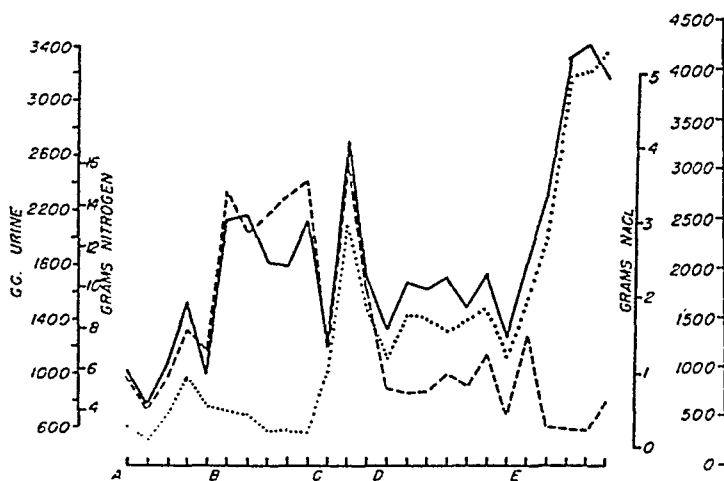
Fig. 3   Fig. 4

Fig. 3. Dog with diabetes insipidus. Parallelism between nitrogen excretion and urine volume when salt intake is low, and between NaCl excretion and urine volume when salt intake is high. Solid line, urine volume. Broken line, nitrogen excretion. Dotted line, chloride excretion as NaCl. A, low nitrogen, low salt diet; B, high nitrogen, low salt diet; C, NaCl intake increased; D, nitrogen intake decreased; E, low nitrogen, high salt diet. Abscissae, 24-hour periods.
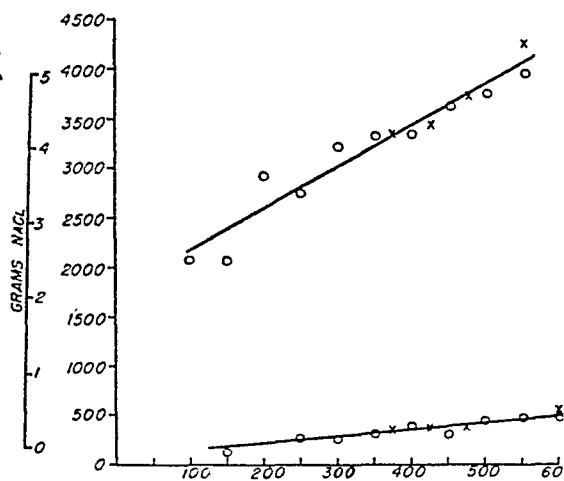
Fig. 4. Relation between urine volume and daily excretion of osmotically active substances. Ordinates, average daily urine volume, cc. Abscissae, sum of chloride and nitrogen excretions, the Cl expressed as os-millimols of NaCl, and the nitrogen expressed as os-millimols of urea. Circles, animals on constant chloride but variable nitrogen intake; crosses, animals on constant nitrogen but variable chloride intake. Lower line, normal dog; upper, dog with diabetes insipidus. The lower line is based on 82, and the upper on 80, daily observations. Straight lines were calculated by the method of least squares.

that there are other osmotically active substances excreted, but under the conditions of these experiments, the substances determined must represent the greater portion of the total osmotic activity of the urine, and as the sodium chloride and nitrogen are varied within the rather wide range shown, so does the total excretion of osmotically active particles vary. The difference in slope, as well as in height, of the lines representing the data from the normal and from the d.i. dog, is an expression of the divergent concentrating abilities of the kidneys of the two dogs. The finding here in the normal dog is somewhat at variance with our data for the normal cat (see above), for in the cat, adding nitrogen to the diet merely increased

[1] The term "os-millimol" is employed here in the same sense as used by Gamble et al. (11).

the concentration of total nitrogen in the urine, without appreciable effect on the urine volume. Gamble (11) reported that in the normal rat, less water was required to excrete urea added to a basal diet than an osmotically equivalent amount of added salt. Our data would make it appear that while the normal cat apparently resembles the rat in that particular, the dog does not. In the presence of d.i., neither the dog nor the cat behave like the normal rat.

Nitrogen partition experiments. Various fractions of the total urinary nitrogen were determined as shown in table 3. Control values were obtained on 4 d.i. cats and 2 normals, and then 2 of the d.i. cats and one of the normals were placed on high nitrogen diet, while the other animals in the experiment were placed on a low nitrogen diet. Finally, 2 of the d.i. cats and one normal were given Pitressin injections, 3 units 3 times daily, while on the stock diet. There is no evidence

TABLE 3

Urinary nitrogen partition in diabetes insipidus and normal cats

Values shown are averages for 24-hour periods

PROCEDURE	KIND OF CAT	NUMBER OF CATS	LENGTH OF PERIOD	TOTAL N	UREA N	AMMONIA N	URIC ACID N	CREATININE N	UN-DETERMINED N
			days	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Control period	d.i.	4	10	2.66	2.31	0.15	0.0028	0.045	0.15
	Normal	2	10	2.66	2.19	0.13	0.0025	0.049	0.29
High-nitrogen diet	d.i.	2	4	3.50	3.29	0.13	0.0025	0.049	0.03
	Normal	1	4	3.64	2.90	0.18	0.0020	0.055	0.50
Low-nitrogen	d.i.	2	4	1.27	1.13	0.08	0.0010	0.049	0.01
	Normal	1	4	2.17	1.99	0.09	0.0016	0.052	0.04
Pitressin injections	d.i.	2	3	2.31	2.12	0.12		0.037	0.03
	Normal	1	3	2.21	2.04	0.09	0.0020	0.052	0.03

from these experiments that there is any difference between the d.i. and the normal cats in nitrogen metabolism. The percentage of the total nitrogen to be found in each of the various fractions determined is essentially the same in both groups; the small differences shown are within the range of the individual daily variations. The nitrogen partition did not appear to be significantly altered by the diets used, nor by Pitressin injections.

Glomerular filtration and tubular reabsorption as affected by dietary changes. Since daily urine volumes reflect changes in nitrogen intake, it is of interest to determine whether the urinary variations are accompanied by alterations of glomerular filtration, tubular reabsorption of water, or both. Therefore, creatinine clearance determinations were made on 2 d.i. and 2 normal dogs under various dietary conditions. It has been shown by others (12–16) that urea and xylose clearances in dogs are elevated by high protein diets, and depressed by low protein diets. Hiatt and Hiatt (17) demonstrated a post-prandial increase in

glomerular filtration (creatinine clearance) and renal plasma flow (diodrast clearance) in the harbor seal, and gave evidence that proteins were the active agents. The plan of our experiments, however, differed somewhat from any of these. Briefly, it was a follows: the animals were placed on a stock diet of dog biscuits; when the urine volumes had assumed their characteristic levels for these diets, the creatinine clearance was measured, usually about 18 hours after the last feeding, except in one dog (no. 4), which was consistently run 8 hours after feeding. At least two control determinations were made on each animal on different days, three clearance periods constituting one determination. The

TABLE 4

Effect of dietary changes on creatinine clearance

(All clearances are post-absorptive except the one indicated otherwise)

DOG NUMBER	KIND OF ANIMAL	DIET	CREATININE CLEARANCE	URINE FLOW DURING CLEAR-ANCE PERIOD	CREATININE U/P
			cc./min.	*cc./min.*	
1	Normal	Control	53.4	0.28	223
		High-nitrogen	47.2	0.27	179
		3-day fast	51.5	0.15	353
		4½ hours after high-N meal	70.4	0.35	203
2	Normal	Control	45.9	0.18	249
		4-day fast	32.0	0.10	328
		Low-nitrogen	46.4	0.20	228
3	d.i.	Control	57.0	2.92	20.2
		4-day fast	52.4	1.82	29.1
		5-day fast	50.6	1.18	42.8
		Low-nitrogen	53.4	1.78	30.3
		High-nitrogen	59.0	3.84	15.5
4	d.i.	Control	49.4	3.97	12.5
		Low-nitrogen	40.3	1.64	24.3
		High-nitrogen	51.3	4.78	10.8

animal was then fasted, or placed on an experimental diet, and after a varying number of days, when the urine volume appeared to be established at a new level, the creatinine clearance was again determined. The high-nitrogen diets we have used were not as high in protein content as the meat diets used by Jolliffe and Smith (13) or by Shannon *et al.* (16), and the variations in clearance we observed were not as great. It was not our purpose to obtain maximum increases in glomerular filtration by excessive protein intakes, but rather to observe whatever alterations in glomerular filtration and tubular reabsorption might be found to accompany the changes in degree of polyuria associated with the different diets.

The results are summarized in table 4. It is clear from these data that changes in tubular reabsorption are much more marked than are those in glomer-

ular filtration. Only in a fast of 4 to 5 days' duration was there any consistently observed diminution of creatinine clearance. The changes in filtration which occurred post-absorptively following a high-nitrogen or low-nitrogen diet are (with the possible exception of the low-nitrogen clearance in dog 4) within the range of variation obtained in the various control observations. On the other hand, tubular reabsorption of water, as shown by the creatinine U/P ratios, varies considerably on the different diets. In all the experiments except one (low-nitrogen in dog 2), tubular reabsorption of water is higher than the control during fasting or low-nitrogen diet, and lower than the control during high-nitrogen diet. The result is as marked in the d.i. as in the normal dogs. It is consistent with the observation (see above) that the urine volume varies with the number of osmotically active particles excreted. On a high nitrogen diet, there are more such particles present in the tubular fluid to hold water within the tubule by osmotic attraction, and hence the percentage reabsorption of water is less. On the low-nitrogen diet, or during fasting, the converse holds. This effect is more or less independent of the titer of antidiuretic substance present, and hence is seen in both the normal and the d.i. animals. This interpretation is consistent with the report of Shannon (18) that the mechanism of water reabsorption is the same in normal and in d.i. dogs. The one example included in table 4 of a post-prandial but not post-absorptive clearance (dog 1) is an illustration of the well-known fact that glomerular filtration is augmented during intestinal absorption of a meal, especially of a high protein meal. This is comparable with the experiments of Hiatt and Hiatt (17). The rest of the figures in the table, however, clearly show that the effect on tubular reabsorption of water of varying the nitrogen intake is not confined to the period during which the meal is being absorbed, even though the glomerular filtration may return to normal after absorption is completed.

SUMMARY

Previous work from this and other laboratories has demonstrated that in diabetes insipidus there is a deficiency in ability to concentrate sodium chloride in the urine. The present work shows that a similar deficiency exists with regard to nitrogenous compounds. Indeed, the latter appear to be relatively more important than the former in the maintenance of the polyuria under ordinary dietary conditions, because nitrogenous substances form the major portion of the total solids of the urine. The polyuria may be markedly reduced in dogs or cats with experimental diabetes insipidus on a nearly constant chloride intake by reducing the nitrogen content of the diet, or may be exaggerated by increasing the nitrogen intake. This effect is independent of the caloric content of the diet. The polyuria parallels the sodium chloride intake even on a low protein diet, if NaCl is fed in excessive amounts, but not unless that salt is present in amount exceeding that found in ordinary diets. In general, the polyuria varies directly with the sum of the osmotic activities of the NaCl and nitrogenous compounds in the urine. Thus, in order to reduce the polyuria of diabetes insipidus by dietary means alone, both the nitrogen and salt intakes must be kept low. Nitrogen

partition determinations show no evidence of any abnormality in nitrogen metabolism in diabetes insipidus. Creatinine clearance studies show that the changes in urine volume accompanying alterations in protein intake extend into the postabsorptive period, after the glomerular filtration has returned to the level observed postabsorptively on a control diet. Postabsorptive tubular reabsorption of water, however, is greater on a low nitrogen diet, and less on a high protein diet, than the control. This is interpreted on the basis of the osmotic effect of the nitrogenous substances present in the tubular fluid.

REFERENCES

(1) WHITE, H. L. AND T. FINDLEY, JR. J. Clin. Investigation 18: 377, 1939.
(2) KARLSON, S. AND B. NORBERG. Acta med. Scand. 88: 585, 1936.
(3) DEBRE, R., J. MARIE, D. NACHMANSOHN AND J. BERNARD. Bull. et Mem. Soc. med. d. Hop. de Paris 52: 967, 1936.
(4) SWANN, H. G. AND B. J. PENNER. Endocrinology 24: 253, 1939.
(5) SWANN, H. G. Endocrinology 25: 288, 1939.
(6) SWANN, H. G. This Journal 126: 341, 1939.
(7) WINTER, C. A., D. G. SATTLER AND W. R. INGRAM. This Journal 131: 363, 1940.
(8) FISHER, C., W. R. INGRAM AND S. W. RANSON. Diabetes insipidus. Edwards Bros., Ann Arbor, 1938.
(9) BING, F. A. AND L. B. MENDEL. This Journal 98: 169, 1931.
(10) LUSK, G. The fundamental basis of nutrition. Yale University Press, ed. 2, 1923.
(11) GAMBLE, J. L., C. F. McKHANN, A. M. BUTLER AND E. TUTHILL. This Journal 109: 139, 1934.
(12) JOLLIFFE, N. AND H. W. SMITH. This Journal 98: 572, 1931.
(13) JOLLIFFE, N. AND H. W. SMITH. This Journal 99: 101, 1931.
(14) VAN SLYKE, D. D., C. P. RHOADS, A. HILLER AND A. ALVING. This Journal 110: 387, 1934.
(15) PITTS, R. F. J. Nutrition 9: 657, 1935.
(16) SHANNON, J. A., N. JOLLIFFE AND H. W. SMITH. This Journal 101: 625, 1932.
(17) HIATT, E. P. AND R. B. HIATT. J. Cell. and Comp. Physiol., 19: 221, 1942.
(18) SHANNON, J. A. J. Exper. Med. 76: 371, 1942.
(19) INGRAM, W. R. AND C. A. WINTER. This Journal 122: 143, 1938.

OBSERVATIONS ON THE POLYURIA PRODUCED BY DESOXYCORTICOSTERONE ACETATE

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A syndrome resembling diabetes insipidus develops in dogs during daily administration of desoxycorticosterone acetate (1–5). Britton and his co-workers (6–9) and Mulinos *et al.* (3) have developed the hypothesis that the adrenocortical hormone and the posterior lobe antidiuretic hormone are physiological antagonists; according to this view, diabetes insipidus may be explained by the unmasking of the diuretic effect of the adrenal cortex when there is a deficiency of posterior lobe secretion. We have for several years maintained a colony of animals with experimental diabetes insipidus (d.i.), and we have sought to determine the effect of injections of corticoadrenal extract and of desoxycorticosterone acetate (DCA) on these animals, as well as to compare the polyuric syndrome produced by such injections in normal animals with that produced by pituitary stalk section.

METHODS. Both cats and dogs have been used in these experiments. The animals were kept in metabolism cages, and measurements of water intake and urine volume were made daily; water was allowed *ad libitum*. Experimental d.i. was produced by pituitary stalk section, leaving the anterior lobe intact. All injections were made subcutaneously. Urinary chloride was determined by the method previously described (10); nitrogen was determined by Nesslerization of a microkjeldahl digest, and reading in a Klett-Summerson photoelectric colorimeter. Postabsorptive plasma creatinine clearances were obtained for some of the dogs, after priming with about 0.2 gram of creatinine per kilogram of body weight by stomach tube about two hours before the determination. Three clearance periods of approximately twenty minutes' duration each were obtained, and the average value for the three periods recorded. Color was developed by the Jaffé reaction, and read in the photoelectric colorimeter.

OBSERVATIONS. *Effect of cortin and of desoxycorticosterone acetate on normal and on d.i. cats.* Cats respond promptly to posterior lobe denervation by increasing the urine volume to four or five times the normal. We therefore thought that if the polyuria of d.i. is due to the diuretic effect of adrenocortical substances, the cat might be a good animal for demonstrating it. A d.i. cat with an average urine volume of about 500 cc. daily was injected with 2 cc. of adrenal cortex extract[1] twice daily for six days. The average urine volume for the six days was 480 cc., and at no time was there any evidence in the 24-hour urine volume of any

diuretic effect of the extract. This result was typical of 7 animals (6 d.i. and 1 normal) to which cortical extract was administered, although in 3 of them the dosage was as high as 8 cc. daily, divided into two injections. Throughout these experiments, the cats were on a constant diet of 80 grams ground raw lean beef and 100 cc. milk, containing about 0.238 gram of Cl as NaCl in a daily portion.

Such results do not necessarily prove that cortical hormone has no diuretic effect in the cat, for the extract was administered in an aqueous medium, and it is quite possible that the cortin was absorbed so promptly that the effect would not show up in a 24-hour urine collection. Furthermore, Mulinos et al. (3) reported that the effect of DCA in dogs is greater if the animals were on a diet containing considerable quantities of salt. We therefore tried injecting DCA in oil, and changed the diet of the cats to 100 grams of canned dog food and 100 cc. of milk; the total Cl as NaCl in the diet was about 0.8 gram daily.

Ragan et al. (2) and Ferrebee et al. (4) gave their dogs 25 mgm. of DCA a day, but Moehlig and Jaffe (5) reported that 5 to 10 mgm. daily would increase the water exchange of dogs, while Mulinos et al. (3) found that the polyuria could be developed by as little as 2 to 4 mgm. daily. We started two normal cats on 15 mgm. daily, gradually increasing the dosage to 30 mgm., continuing the injections for a total of 11 days, with no evidence of diuretic effect at any time. Two d.i. cats were then started on 30 mgm. daily, but there was no increase in the polyuria already present. Three units of aqueous pitressin injected 3 times daily promptly reduced the urine volume of the d.i. cats receiving DCA nearly to a level characteristic of a normal cat. This is about the minimal subcutaneous dose required to control the polyuria of a d.i. cat (10), and the reduction in urine volume in this instance was as complete and as prompt as we have repeatedly observed in d.i. cats not receiving DCA. Protocols of two typical experiments are given in table 1.

From these results we must conclude that in the amounts used, at least, neither adrenocortical hormone nor DCA will produce a syndrome resembling d.i. in normal cats, nor will these substances increase either the severity of d.i. already present, or the dosage of pitressin required to control it. The cats received from 6 to 10 mgm. of DCA per kgm. of body weight daily, a dosage about 17 times as great on a body weight basis as the average we used on dogs.

Effect of DCA injections on normal and on d.i. dogs. Since we were unable to produce the polyuric syndrome with DCA in cats, we began injections in 4 d.i. and 3 normal dogs. The animals weighed from 11 kgm. to 22 kgm., and were fed from 200 grams to 300 grams of commercial dog biscuit daily. On this diet, they excreted 6 to 8 grams of nitrogen and 1.3 to 2 grams of Cl as NaCl in the urine daily. After a control period, the animals were injected with 2.5 to 10 mgm. of DCA daily. In confirmation of the reports of others (2–5), all the normal dogs developed a polyuria. These animals excreted an average of 150 cc. to 200 cc. of urine before the injections began; on the fourth day of injections, in all the normal animals the urine volume suddenly increased to about 400 cc. daily, and thereafter increased gradually to between 1 and 1.5 liters daily. None of our dogs or cats at any time exhibited any overt signs of the muscular weakness

described by others (2, 4, 5) as a result of DCA injections, although two dogs were continued on the 10 mgm. daily dose for three months each.

The diuretic effect of the injections was seen more promptly in the d.i. dogs than in the normals. Dog 1, a d.i. animal weighing 21 kgm., averaged about 2650 cc. of urine daily immediately before the injections, and excreted 4070 cc. the first day the hormone was administered. Another d.i. dog (no. 3) increased from an average of 2060 cc. to 2900 cc. the first day. Thereafter the increase was gradual, so that at the end of a week the daily volumes in the two animals were 5500 cc. and 3500 cc., respectively. Dog 1 had a daily urine output of about 7 liters after one month on the hormone, and after three months, when the

TABLE 1

Absence of effect of desoxycorticosterone acetate on water exchange of cats

| | CAT WITH DIABETES INSIPIDUS | | | | | NORMAL CAT | | | | |
Date	Total water intake	Urine volume	Total N in urine	Cl as NaCl in urine	Injections	Date	Total water intake	Urine volume	Total N in urine	Cl as NaCl in urine	Injections
	cc.	cc.	grams	grams			cc.	cc.	grams	grams	
11/5	600	490	1.61	0.905	None	10/25	180	140	2.45	0.991	None
6	590	470	1.50	0.712	None	26	180	140	2.33	0.764	None
7	630	515	1.52	1.015	None	27	180	100	1.83	0.730	None
8	580	480	1.69	0.838	None	28	180	105	2.06	0.840	None
9	570	485	1.49	0.947	None						
10	560	415	1.30		None	29	185	70	1.12	0.327	15 mgm. DCA
						30	180	60	1.33	0.280	15 mgm. DCA
11	640	565	1.88	0.955	30 mgm. DCA	31	180	175	3.94	1.625	15 mgm. DCA
12	660	555	1.53	0.920	30 mgm. DCA	11/1	180	150	2.22	1.060	15 mgm. DCA
13					30 mgm. DCA	2	180	105	2.07	1.010	15 mgm. DCA
14	630	490	1.53	0.871	30 mgm. DCA	3	180	105	1.63	0.830	20 mgm. DCA
15	615	510	1.79	0.859	30 mgm. DCA	4	180	80	1.37	0.558	20 mgm. DCA
16	625	525	1.96	0.815	30 mgm. DCA	5	180	135	2.11	0.944	20 mgm. DCA
						6	180	145	2.06	0.336	30 mgm. DCA
17	290	165	1.34	0.488	30 mgm. DCA + 9 units pitressin	7	180	155	1.90	0.620	30 mgm. DCA
						8	195	75	1.44	0.296	30 mgm. DCA
18	265	220	2.14	0.754	30 mgm. DCA + 9 units pitressin	9	180	175	2.13	0.550	None
						10	180	70	1.34	0.495	None
						11	180	130	1.90	0.885	None
19	555	410	1.38	0.433	None						
20	400	500	1.38	1.015	None						
21	525	455	1.69	0.740	None						

injections were discontinued, she was averaging nearly 9 liters daily. On the last day of injection, this animal excreted 9645 cc. of urine; three days later, the daily output had dropped to about 3 liters, or about the same level as before the injections began.

Mulinos *et al.* (3) thought that the polydipsia appeared before the polyuria, but this did not seem to be the case in any of our 7 dogs. The two phenomena appeared to keep pace with each other, so that if one calculates the water balance (total intake − urine volume) before and after the start of the injections, there appears to be no significant difference, although the day-to-day variations are so large that one cannot be certain on that point (table 2).

Effect of variations in salt and nitrogen intake. In view of the well-known inability of the d.i. kidney to form a concentrated urine, and the consequent influence of dietary changes on the volume of the polyuria, we have fed various diets to dogs receiving DCA injections. Results of such experiments on one d.i. and one normal dog are summarized in table 3. It will be seen that the degree of the polyuria varies somewhat with the amount of nitrogen and salt which the kidney is required to excrete. In this respect, the non-d.i. animal receiving DCA resembles the ordinary d.i. dog; the difference appears to be one of degree. The d.i. animal shown in this table is the same dog as shown in figure 2 of reference 13. A comparison of the two results shows that when the animal was on DCA, reduction in nitrogen intake did not reduce the polyuria as markedly as a similar

TABLE 2

Effect of desoxycorticosterone acetate injections in dogs

	DOG WITH DIABETES INSIPIDUS					NORMAL DOG				
Date	Water in-take	Urine vol-ume	Total N in urine	Cl as NaCl in urine	Injections	Date	Water in-take	Urine vol-ume	Total N in urine	Cl as NaCl in urine	Injections
	cc.	cc.	grams	grams			cc.	cc.	grams	grams	
12/10	2930	2370	6.82	2.05	None	12/10	470	250	6.43	2.33	None
11	3190	2870	9.77	2.01	None	11	530	140	5.85	1.83	None
12	3470	2710	8.25	1.86	None	12	510	190	6.68	2.03	None
13	2300	2680	7.88	1.76	None	13	630	130	3.82	0.69	None
14	3610	2520	7.19	1.78	None	14	730	200	9.75	1.78	None
15	3300	2800	8.61	2.17	None	15	530	120	4.38	0.93	None
16	4580	4070	8.12	1.10	10 mgm. DCA	16	650	140	6.17	1.20	10 mgm. DCA
17	5630	4920	6.81	1.05	10 mgm. DCA	17	720	380	6.37	3.20	10 mgm. DCA
18	4990	3950	6.65	0.64	10 mgm. DCA	18	850	140	4.46	0.81	10 mgm. DCA
19	4890	4620	8.57	2.71	10 mgm. DCA	19	820	480	9.85	3.24	10 mgm. DCA
20	5540	5000	8.75	1.54	10 mgm. DCA	20	960	400	7.97	1.52	10 mgm. DCA
21	5960	5440	8.74	1.89	10 mgm. DCA	21	860	380	8.10	2.10	10 mgm. DCA
22	7100	5500	8.38	3.18	10 mgm. DCA	22	1060	430	8.57	2.19	10 mgm. DCA
23	6930	6580	8.65	1.44	10 mgm. DCA	23	880	250	6.92	1.12	10 mgm. DCA
24	7910	7230	8.51	3.33	10 mgm. DCA	24	1170	560	8.06	1.82	10 mgm. DCA
25	7410	6800	8.39	2.52	10 mgm. DCA	25	1320	540	6.66	2.68	10 mgm. DCA
26	8400	7880	8.51	1.62	10 mgm. DCA	26					10 mgm. DCA
27	8070	7810	8.23	2.55	10 mgm. DCA	27	1145	710	7.07	2.60	10 mgm. DCA

experiment did while the animal was not receiving the injections. Indeed, as is shown in table 3, even a three-day fast while the animal was on DCA left her with a polyuria of 3 liters; the same dog when fasted before the injections began promptly reduced her urine output to 800 cc. So far as the feeding experiments go, then, DCA appears to exaggerate d.i. when it is already present, and a normal animal receiving the injections acts like a dog with mild d.i.

Effect of pitressin injections on normal and on d.i. dogs receiving DCA. Ragan *et al.* (2) reported that as much as 3 cc. daily of pitressin did not control the polyuria of a dog receiving 25 mgm. of DCA and 8.5 grams of NaCl daily. However, they did not state whether or not the pitressin was given in divided doses (as it must be if aqueous pitressin is used), and furthermore it is well known that

pitressin will not control a salt diuresis, and their animals were on a high salt intake. On the other hand, Mulinos *et al.* (3) found that 11 units of pitressin in oil markedly reduced the polyuria of dogs receiving only 2 to 4 mgm. of DCA daily. We have tried two levels of pitressin in two dogs, one d.i. and one non-d.i., receiving the 10 mgm. dosage of DCA. Five units of pitressin tannate in oil was administered in two daily doses of 2.5 units each; in the non-d.i. dog, the urine volume was reduced from about 1200 cc. to about 900 cc., and in the d.i. dog from 6500 cc. to about 2800 cc. This is a much smaller effect than is ordinarily seen in injecting pitressin tannate in oil during d.i., so we next tried injecting 35 units over a period of 4 days; this reduced the polyuria of both animals to

TABLE 3

Effect of dietary changes on the urinary excretion of dogs receiving injections of desoxycorticosterone acetate

NO. OF DAYS	DIET	INTAKE			URINARY EXCRETION				
						Nitrogen		Chloride as NaCl	
		Water	Nitrogen	Chloride as NaCl	Volume	Concentration	Total	Concentration	Total
d.i. dog									
		cc.	grams	grams	cc.	mgm. per cent	grams	mgm. per cent	grams
9	Control	7982	11.81	2.13	7233	104	7.54	31.9	2.31
8	Low-N	6171	2.95	2.03	5694	63	3.61	39.4	2.24
9	Low-N, Low salt	4674	2.95	0.53	4169	82	3.41	16.1	0.67
3	Fast	3077	0	0	3020	93	2.81	16.6	0.50
5	High-N	8880	18.01	2.18	8307	159	13.23	34.4	2.86
Normal dog									
9	Control	1262	11.81	2.13	699	895	6.26	291	2.03
8	Low-N	905	2.95	2.03	574	537	3.08	370	2.12
9	Low-N, Low salt	844	2.95	0.53	457	656	3.00	103	0.47
3	Fast	640	0	0	407	492	2.00	103	0.42
5	High-N	1715	18.01	2.18	1198	1043	12.50	186	2.23

about 800 cc. In contrast to this mild effect, the same d.i. animal was again injected with pitressin tannate in oil 43 days after DCA was discontinued. This time, 5 units in a single injection rendered the animal completely anuric for two days, although immediately before the injection the animal was excreting an average of about 4 liters of urine a day. It is clear, then, that DCA greatly reduces the antidiuretic potency of pitressin in d.i. dogs, in marked contrast to the effect in d.i. cats.

Effect of DCA on glomerular filtration and tubular reabsorption of water. It is generally held that the polyuria of d.i. is due to defective tubular reabsorption of water; in well-established d.i., glomerular filtration does not differ significantly

from normal (table 4; and references 11, 12 and 19). In order to compare kidney function in the polyuria of d.i. with that in DCA polyuria, tests were run on 4 d.i. and 2 normal dogs, before DCA injections, and during the course of administration. As shown in the table, all clearances obtained during the injection period were taken at a time when the urine flow was significantly above that seen before injections began. Each figure in the table is the average for 3 to 6 clearance periods.

In the dog, creatinine clearance is generally considered to be a measure of glomerular filtration, while the creatinine U/P ratio (concentration of creatinine in the urine divided by that in the plasma) is an index of tubular reabsorption of

TABLE 4

Effect of desoxycorticosterone acetate on glomerular filtration and tubular reabsorption

DOG NUMBER	KIND OF ANIMAL	WITHOUT DESOXYCORTICOSTERONE			DURING INJECTION PERIOD			DAILY DOSAGE OF DCA
		Plasma creatinine clearance	Ave. urine flow during experiment	Creatinine U/P	Plasma creatinine clearance	Ave. urine flow during experiment	Creatinine U/P	
		cc./min.	cc./min.		cc./min.	cc./min.		mgm.
1	d.i.	51.5	2.95	17.7	63.1	3.80	16.5	10
					73.4	4.32	17.1	10
4	Normal	48.5	0.22	271	54.0	0.58	93	5
		58.3	0.34	174	49.5	0.69	77	5
3	d.i.	45.0	0.98	36	43.5	2.95	15.0	5
					45.0	2.85	16.0	5
5	Normal	42.2	0.17	243	61.0	0.51	121	10
		49.5	0.19	254	63.0	0.75	89	10
6	d.i.	57.8	2.90	21	70.8	9.03	7.8	10
					65.8	5.27	12.5	10
7	d.i.	48.0	3.84	12.6	56.1	5.63	10.0	2.5
		50.8	4.10	12.4	58.0	6.67	8.7	5
					60.4	6.27	9.9	10

water, since $\dfrac{100\,(U/P - 1)}{U/P} = $ per cent of filtered water reabsorbed in the tubule. The higher the U/P ratio, the more efficient is the tubular reabsorption of water. Increased urine volume might be the result of more rapid filtration without a corresponding increase in the percentage of water reabsorbed, or it might be due to a decrease in the rate of reabsorption. The data show that DCA injections diminish the tubular reabsorption of water. This effect appears to be very small in dog 1, but is very evident in all the other animals. The effect of DCA on glomerular filtration appears to depend on the dosage; in every case where 10 mgm. a day was injected, creatinine clearance was found to be significantly elevated; with the smaller doses, there was little or no effect.

DISCUSSION. The hypothesis that the polyuria of d.i. is due to the diuretic effect of corticoadrenal hormone when it is released from the restraining influence of the antidiuretic hormone of the posterior lobe rests principally upon the following observations: 1. DCA injections have a diuretic effect in the intact rat; hypophysectomy is variously said to augment (20) or prevent (21) the effect. 2. The transient polyuria observed in the rat after hypophysectomy does not appear if the adrenals are removed simultaneously with the pituitary (14). 3. In short courses of injections in rats, DCA and posterior lobe extracts have opposite effects on excretion of water, chloride, and sodium (9). 4. In either adrenalectomized or intact opossums, cortin injections augment a water diuresis, giving a more dilute urine, while posterior lobe extract inhibits water diuresis, giving a more concentrated urine (6). 5. DCA injections in dogs lead to an augmented excretion of urine, with specific gravity lower than normal. 6. Some sort of antagonism between DCA and the antidiuretic hormone is indicated by the observation that pitressin is less effective than usual in controlling d.i. in dogs, while DCA is being administered.

On the other hand, merely demonstrating a diuretic effect of cortical extract or of DCA when injected under suitable conditions does not prove that the normal secretion of the intact adrenals is the diuretic agent in d.i. Indeed, there are several observations which speak definitely against such a hypothesis. Schweizer et al. (15) found that adrenotropic hormone in doses sufficient to maintain adrenal size above normal levels failed to prevent the cessation of the transient polyuria of hypophysectomized rats. Furthermore, neither adrenotropic hormone nor cortical extract would reinstitute the polyuria after it had subsided. Both Ragan et al. (2) and Mulinos et al. (3) reported that dogs with DCA polyuria stood water restriction without symptoms of dehydration. This is in marked contrast to the effect of water restriction in d.i.

If the polyuria of d.i. were due to the adrenocortical secretion, one might expect that adrenalectomy would "cure" the d.i., but in the cat, at least, such is not the case (16). Although the polyuria is diminished after adrenal removal, this appears to be merely a reflection of lessened water intake, for the latter decreases even more than the urine volume, and the specific gravity of the urine shows that after adrenalectomy of a d.i. cat, d.i. still exists. Furthermore, the maintenance of a high degree of polyuria in adrenalectomized d.i. cats during salt feeding offers evidence that cortin is not essential for the occurrence of d.i. in the cat (18). It is difficult to believe that the mechanism for the production of the polyuria of d.i. is different in the cat than in other species. Our failure to produce a polyuria in normal cats with injections of relatively large doses of DCA, or to increase a d.i. already present, or to increase the pitressin requirement in a d.i. cat, does not lend support to the hypothesis under discussion.

Ragan et al. (2) and Mulinos et al. (3) emphasized the relation between salt intake and the polyuria produced by DCA. The former reported that they were unable to produce the polyuria in rats, unless the animals were placed on a high salt diet; the latter found that in dogs receiving 2 to 4 mgm. of DCA daily, the polyuria depended largely upon the salt present in the diet. Although it is

true that in ordinary d.i., large amounts of salt will increase the polyuria, it has been shown (13, 17) that in d.i. a good polyuria with low specific gravity can exist on a low salt intake, or even a practically salt-free diet.

The polyuria produced by DCA injections is of a relatively low order of magnitude, even in dogs, and never approaches the level seen in a good d.i. preparation, even though the hormone is administered in amounts undoubtedly far above physiological levels. Our finding that DCA injections retard tubular reabsorption of water in dogs does not prove that the adrenal cortex is responsible for the similar phenomenon seen in d.i. We may merely have succeeded in demonstrating an interesting pharmacodynamic effect of the hormone when it is present in abnormally high concentrations. We must conclude that although the hypothesis of the participation of the adrenal cortex in the establishment of the polyuria of d.i. has many attractive features, we must regard it as being far from proven.

SUMMARY

In the dog, injection of 2.5 mgm. to 10 mgm. daily of desoxycorticosterone acetate markedly increases the severity of diabetes insipidus when it is present. Normal dogs receiving such injections develop a syndrome which resembles a very mild diabetes insipidus. Pitressin only partially controls desoxycorticosterone polyuria, even if given in dosage several times that required to render a dog with diabetes insipidus non-polyuric. During administration of desoxycorticosterone acetate, reduction of protein intake while the salt intake is kept constant reduces the polyuria, but the change in urine volume is less marked than when a similar experiment is performed on an ordinary d.i. dog. Increase in nitrogen intake is markedly effective in increasing the polyuria. Creatinine clearance studies show that, both in normal and in d.i. dogs, desoxycorticosterone acetate reduces tubular reabsorption of water. Dosages as large as 10 mgm. daily also increase glomerular filtration, but smaller doses have little or no effect. In contrast to the results in dogs, injections of desoxycorticosterone acetate in dosages up to 30 mgm. daily fail to increase the daily water exchange of normal cats. In cats with experimental diabetes insipidus, similar injections increase neither the severity of the d.i., nor the dosage of pitressin required to relieve it. The relation of these findings to the hypothesis that the adrenocortical hormone and the posterior lobe antidiuretic hormone are physiological antagonists, is discussed.

REFERENCES

(1) KUHLMANN, D., C. RAGAN, J. W. FERREBEE, D. W. ATCHLEY AND R. F. LOEB. Science 90: 496, 1939.

(2) RAGAN, C., J. W. FERREBEE, P. PHYFE, D. W. ATCHLEY AND R. F. LOEB. This Journal 131: 73, 1940.

(3) MULINOS, M. G., C. L. SPINGARN AND M. E. LOJKIN. This Journal 135: 102, 1941.

(4) FERREBEE, J. W., D. PARKER, W. H. CARNES, M. K. GERITY, D. W. ATCHLEY AND R. F. LOEB. This Journal 135: 230, 1941.

(5) MOEHLIG, R. C. AND L. JAFFE. J. Lab. and Clin. Med. 27: 1009, 1942.

(6) SILVETTE, H. AND S. W. BRITTON. This Journal 123: 630, 1938.

(7) SILVETTE, H. AND S. W. BRITTON. Science 88: 150, 1938.

(8) BRITTON, S. W. AND E. L. COREY. Science 93: 405, 1941.

(9) COREY, E. L. AND S. W. BRITTON. This Journal 133: 511, 1941.

(10) WINTER, C. A., W. R. INGRAM, E. G. GROSS AND D. G. SATTLER. Endocrinology 28: 535, 1941.

(11) WHITE, H. L. AND P. HEINBECKER. This Journal 123: 566, 1938.

(12) HARE, K. Personal communication.

(13) WINTER, C. A., W. R. INGRAM AND R. C. EATON. This Journal 139: 700, 1943.

(14) COREY, E. L., H. SILVETTE AND S. W. BRITTON. This Journal 125: 644, 1939.

(15) SCHWEIZER, M., R. GAUNT, W. O. NELSON AND E. LOOMIS. Anat. Rec. 72: Sup. 122, 1938.

(16) INGRAM, W. R. AND C. A. WINTER. This Journal 122: 143, 1938.

(17) WINTER, C. A., D. G. SATTLER AND W. R. INGRAM. This Journal 131: 363, 1940.

(18) WINTER, C. A., W. R. INGRAM AND E. G. GROSS. This Journal 126: P544, 1939.

(19) SHANNON, J. A. J. Exper. Med. 76: 371, 1942.

(20) SELYE, H. AND L. BASSETT. Proc. Soc. Exper. Biol. and Med. 45: 272, 1940.

(21) HARNED, A. S. AND W. O. NELSON. Federation Proc. 2: 19, 1943.

THE EFFECTS OF POTASSIUM ARSENITE (FOWLER'S SOLUTION) ON THE RESPIRATION AND GLYCOLYSIS OF NORMAL AND LEUKEMIC TISSUES, WITH OBSERVATIONS ON THE ACTION OF MENADIONE, 2-METHYL-1, 4 NAPHTHOQUINONE[1]

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The purpose of these experiments was twofold, namely, to study the effect of potassium arsenite on the respiration and glycolysis of normal and leukemic tissues and to attempt to devise a method for increasing the effectiveness of this compound in the treatment of leukemia. The second endeavor has not been successful, but the results are of interest in that they throw light upon the *in vitro* effects of menadione.

The use of potassium arsenite (Fowler's solution) in the treatment of leukemia has been developed particularly by Forkner and his associates (1) and is reviewed in his monograph (2). The drug is useful particularly in cases of chronic myelogenous leukemia, frequently inducing a marked reduction in the leucocyte count and in the leucocytic infiltration of tissues and improving the general clinical condition of the patients. These effects are of interest in that they represent one of the few instances in which chemotherapy is of any value in the treatment of a malignant disease. The mechanisms by which the drug produces these effects are not well understood, although arsenite has long been known to inhibit the respiration of a variety of tissues. Also, the extensive studies of Krebs, Szent-Gyorgyi and their associates (3, 4, 5) have shown that arsenite inhibits keto acid oxidation in kidney, liver, and pigeon breast muscle. Its action on leucocytes and myeloid bone marrow cells, however, and its effect on glycolysis, except for the limited work of Dresel (6), have apparently not been investigated. The following experiments elucidate some of these points.

I. *Effects of Potassium Arsenite on the Respiration and Glycolysis of Normal and Leukemic Tissues. A. Effect on respiration.* In these experiments, the respiration of tissue slices or blood cells was measured in the Warburg apparatus, at 38°C., with and without the addition of potassium arsenite. Sodium arsenite had the same effects. The medium was neutralized serum (7) *i.e.*, serum to which sufficient HCl had been added to maintain the pH at 7.4 in the absence of CO_2 in the gas phase. The duration of the experiments was usually three hours. With most tissues, the depressant effect of arsenite on respiration is progressive during approximately the first hour, after which the respiration is maintained at a nearly constant, though reduced, rate. This latter period has been used in comparing the rate of respiration of the arsenite-poisoned and normal tissues.

The results are shown in table 1, from which it may be seen that the inhibition of respiration in the leukemic cells is of the same order of magnitude as in the normal tissues studied, a selective action of arsenite on leukemic cells not being demonstrable by these methods. The possibility remains that leukemic bone marrow may be more sensitive to the action of arsenite than leukemic circulating leucocytes or tumors; this has not been investigated because of the difficulty of obtaining sufficient quantities of leukemic marrow (without gross contamination with blood) for studies by these methods. Also, arsenite may accumulate *in vivo* in the marrow, although the experiments of Hunter, Kip and Irvine (8) do not suggest this. The clinical effectiveness of arsenite in the treatment of leukemia consequently remains obscure. But the experiments do show that arsenite is a general protoplasmic poison, and any method of inducing a more

TABLE 1

The effect of graded concentrations of potassium arsenite on the respiration of various tissues

TISSUE	% DECREASE IN Q_{O_2} AT KA_sO_2 CONC. OF:			
	1×10^{-3}	2×10^{-4}	1×10^{-4}	1×10^{-5}
Rabbit bone marrow	72 (12)*	56 (8)	41 (5)	11 (2)
Human rib marrow	58 (5)			
Normal human leucocytes			32 (1)	12 (1)
Rabbit exudate leucocytes	77 (2)	38 (1)	30 (1)	
Rabbit kidney	71 (1)	30 (1)	25 (1)	
Rabbit liver	36 (2)			
Rabbit brain		51 (1)		
Rat testis		49 (2)		19 (1)
Human lymphogenous leukemia (blood)	62 (2)	44 (2)	22 (5)	
Human myelogenous leukemia (blood)	62 (7)	49 (2)	33 (5)	
Mouse lymphogenous leukemia (tumor)	72 (3)			
Mouse myelogenous leukemia (tumor)	55 (2)			

* The figures in parentheses represent the number of experiments performed. All measurements were made in duplicate.

specific action on leukemic cells would be welcome. An attempt to accomplish this is described in section II, below.

B. *Effect on glycolysis.* Concomitant with the decrease in respiration induced by arsenite, an increase in aerobic acid production occurs in bone marrow and normal and leukemic leucocytes. A representative manometric experiment demonstrating this manifestation of the Pasteur effect[3] is depicted in figure 1. Lactic acid analyses made by the method of Barker and Summerson (10) disclose that the acid liberated is lactic acid. Keto-acid analyses made by the method of Penrose and Quastel (11) failed to show keto-acid accumulation in these tissues in contrast to that in kidney, liver, and pigeon breast muscle referred to above (3, 4, 5). In this respect, arsenite has a qualitatively different type of action on myeloid tissue than on the other tissues cited, but whether this

[3] The Pasteur effect in bone marrow is characterized by an increase in glycolysis which is reciprocally related to the decrease in respiration occurring at lowered oxygen tensions (9).

is the basis for its selective action on myeloid tissue *in vivo* may be questioned. In concentrations which produce the marked effects on respiration and aerobic glycolysis depicted in figure 1, we find that arsenite inhibits marrow anaerobic glycolysis some 10–20 per cent; the enhancement of aerobic glycolysis is consequently the result of the impaired respiration rather than a direct effect on glycolytic mechanisms *per se*.

II. *Action on Menadione in Counteracting the Metabolic Effects of Arsenite.* Before studying the effects of menadione, the action of glutathione was investigated, because the well-known experiments of Voegtlin and his collaborators (12) suggested that this compound might be useful in protecting normal tissues from the action of arsenite. But our experiments revealed that marrow in its optimal medium (serum) is unable to keep glutathione in the reduced state necessary to
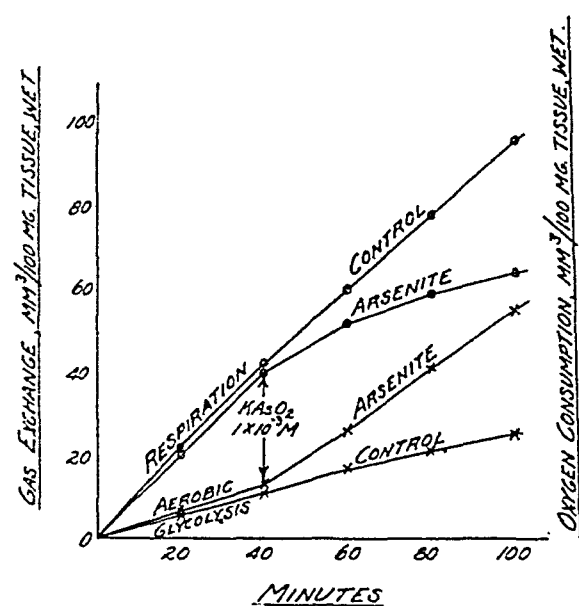
Fig. 1
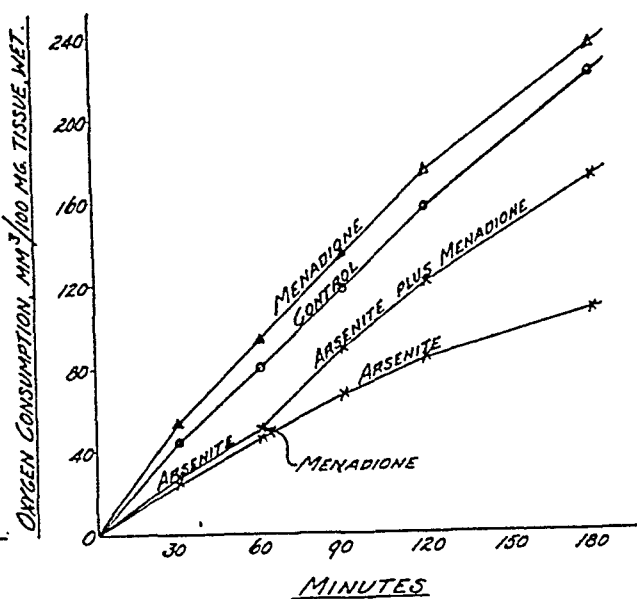
Fig. 2

Fig. 1. Effects of arsenite on rabbit bone marrow respiration and glycolysis.
Fig. 2. Action of menadione in counteracting the effect of arsenite on rabbit bone marrow respiration. Concentrations are 2×10^{-4} M. arsenite and 2.8×10^{-4} M. menadione.

obtain protection. In fact, even in Ringer solution, in which glutathione remains in the sulfhydryl form, no protection against the arsenite effects is obtained with ratios of glutathione:arsenite concentrations as high as 50:1.

The possibility that menadione (2-methyl-1, 4 naphthoquinone) might be of some value in counteracting the effects of arsenite was suggested by the report of Fosdick, Fancher and Calandra (13) that menadione inhibits aerobic glycolysis of bacteria in the saliva. Summerson (14) observed a similar, though less marked, effect on the aerobic glycolysis of exudate leucocytes, in which it also induces a transient increase in oxygen consumption. On both respiration and aerobic glycolysis, therefore, its action is the reverse of that of arsenite. Furthermore, both compounds are known to react with sulfhydryl groups (12, 14, 15). On the basis of these facts, studies were made of the ability of menadione

to counteract the effects of arsenite on marrow respiration and glycolysis. Figure 2 shows the results of a typical experiment in which rabbit bone marrow respiration was measured with and without addition of arsenite, and menadione was added to one of the samples of tissue after it had been poisoned with arsenite for one hour. For the next 45 minutes, the rate of respiration was restored to virtually normal, but decreased to an intermediate rate during the next hour. The increased oxygen consumption of normal tissue treated with menadione is also shown. Description of numerous experiments demonstrating these effects does not appear warranted in view of the findings to be reported below, but the same type of result was also obtained with human rib marrow and with exudate leucocytes. Also, in these tissues, the increase in aerobic glycolysis induced by arsenite is reversed by menadione. The R. Q. of marrow treated with menadione, with or without added arsenite, is the same as that of normal marrow, about 0.96 (16), suggesting that the increased oxygen consumption induced by menadione is a true respiration.[4]

In the hope that menadione would counteract the effects of arsenite on normal but not leukemic cells, this point was investigated using both mouse and human leukemic material. The respiration of 3 myeloid and 3 lymphoid mouse leukemic tumors, kindly supplied by Doctor Furth, was studied with the addition of arsenite and arsenite plus menadione. Only slight reversals (0–30 per cent) of the arsenite depression of respiration were obtained, as compared with an 85 per cent reversal in the case of normal rabbit bone marrow under similar conditions. With suspensions of leukemic leucocytes from 2 cases of human myelogenous leukemia, the reversal of the arsenite depression of respiration averaged 61 per cent.

On the basis of these somewhat encouraging results with the mouse leukemias, Dr. Jacob Furth and Dr. Curtis Flory, of the Department of Pathology, investigated the feasibility of using arsenite plus menadione in the treatment of leukemia in mice. The results of preliminary observations were not encouraging and it was noted that the minimum lethal dose of arsenite was not appreciably elevated by simultaneous administration of menadione. This suggested that the effects of arsenite on tissues other than myeloid tissue might not be counteracted by menadione. Accordingly, tissue respiration experiments were performed with liver and kidney cortex slices and it was found that menadione was completely unable to overcome the depressant effects of arsenite on these organs. In these tissues, as mentioned above, arsenite has a different effect than on myeloid tissue in that keto-acids rather than lactic acid accumulate as the result of the poisoning. It may also be noteworthy that these tissues, unlike myeloid cells, do not normally exhibit aerobic glycolysis.

Finally, to determine whether menadione truly protects marrow cells from

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[4] It should be noted in passing that the concentrations of menadione used in this study are so much greater than those needed to obtain anti-hemorrhagic effects in vitamin K deficiency that no inferences may be drawn from the present experiments with reference to its action as a vitamin K substitute.

the action of arsenite, we have used the supravital staining technique to make direct observations of normal bone marrow cells poisoned with arsenite *in vitro*. The leucocytes and metamyelocytes normally exhibit active amoeboid movement, and in most myelocytes, the granules are in rapid movement, sometimes associated with streaming movements of the cytoplasm. All these types of cellular activity are progressively depressed by arsenite in the concentrations necessary to depress respiration. Lower concentrations are without effect on motility.

Menadione was found to be completely ineffective either in preventing or in restoring this damage to cellular and intracellular motility. In fact, if the greatly impaired motility is regarded as manifesting a toxic effect on the cells (as seems probable), menadione itself is toxic in the concentrations necessary to maintain respiration, for it impairs motility to fully the same extent as arsenite. These observations do not necessarily preclude the possibility of very small doses of menadione and arsenite being of some value therapeutically in leukemia, but do serve to make the outlook less promising than would otherwise be inferred from the respiration studies. Glutathione was also found to be ineffective in preventing or counteracting the arsenite effects on motility.

III. *Mechanism of the Menadione Effects.* This subject has not been investigated fully, but three factors are probably involved. One is the oxidation-reduction potential of the compound. Dr. Otto H. Müller of the Department of Anatomy was kind enough to investigate this point by measuring the oxidation-reduction potential of menadione by the polarographic method (17). He found the value of E_h at pH 7.4 to be +0.035 v. and noted that this potential was intermediate between that of methylene blue, +0.01, and the related compound thionine, +0.05 (18). Subsequently, both these dyes, particularly the latter, were also found to counteract the effects of arsenite on marrow respiration and glycolysis. Like menadione, neither protected the motility of myeloid cells from the action of arsenite although thionine did not of itself impair motility. Methylene blue was quite toxic to motility. The parallelism between the actions of menadione and methylene blue was further strengthened by demonstrating that menadione, like methylene blue (19), markedly increases the respiration of mammalian erythrocytes. Consequently, in studying its effects on leukemic blood cells, the erythrocytes were first removed by differential sedimentation.

A second factor is the possibility of menadione enhancing the oxidation of lactate. de Meio, Kissin and Barron (20) point out that methylene blue particularly increases the respiration of those tissues which exhibit aerobic lactic acid formation and present evidence that lactate is the substrate for the methylene blue effect. Menadione, as mentioned above, counteracts the effects of arsenite on myeloid tissues, in which lactic acid accumulates, but does not do so with liver and kidney cortex, in which there is no accumulation of lactic acid. These facts, plus the similarity of the oxidation-reduction potentials of menadione and methylene blue, suggest that lactate may be oxidized by menadione. This would also account, either wholly or in part, for the effect of menadione in

decreasing the aerobic glycolysis of marrow (present paper), exudate leucocytes (14) and certain bacteria (13).

A third factor affecting the action of menadione may be its ability to react with sulfhydryl groups (12, 15). Phthiocol, 2-methyl-3-hydroxy-1, 4 naphthoquinone, is similar in structure to menadione except for the additional hydroxy group in the 3-position which blocks the reaction of the compound with sulfhydryl groups (15). Phthiocol was found to be completely without effect in counteracting the action of arsenite on marrow. However, the oxidation-reduction potential of this compound at pH 7.4 is −0.200 (21), i.e., so much lower than that of menadione, methylene blue and thionine that it is not possible to conclude whether the failure of phthiocol to counteract the action of arsenite is due to its inability to react with sulphydryl groups, its low oxidation-reduction potential or to both properties, which may themselves be related.

SUMMARY AND CONCLUSIONS

1. Potassium (or sodium) arsenite depresses to much the same extent the respiration of normal rabbit bone marrow, normal human bone marrow and human leukemic leucocytes. This is accompanied by an accumulation of lactic acid in these tissues in contrast to the keto-acid accumulation in liver, kidney and pigeon breast muscle demonstrated by other authors.

2. Menadione increases marrow respiration, decreases aerobic glycolysis and under selected conditions counteracts the effects of arsenite on these metabolic entities.

3. Nevertheless menadione is unable to restore the amoeboid and granular movement of myeloid cells poisoned with arsenite, and of itself impairs these forms of motility.

4. The mechanism of the action of menadione is discussed in relation to its oxidation-reduction potential, its ability to react with sulfhydryl groups and the possibility of its inducing the oxidation of lactate. In several respects, its action *in vitro* is similar to that of methylene blue.

5. Glutathione is ineffective in preventing or counteracting the effects of arsenite on the respiration and motility of marrow cells.

REFERENCES

(1) FORKNER, C. E. Med. Clin. North America **15**: 1057, 1932.
(2) FORKNER, C. E. Leukemia and allied disorders. Macmillan Co., New York, 1938.
(3) KREBS, H. A. Ztschr. f. physiol. Chem. **217**: 191, 1933.
(4) KREBS, H. A. AND JOHNSON. Enzymologia 4: 148, 1937.
(5) SZENT-GYÖRGYI. Ztschr. f. physiol. Chem. **236**: 1, 1935.
(6) DRESEL, K. Biochem. Ztschr. **178**: 70, 1926.
(7) WARREN, C. O. This Journal **128**: 455, 1940.
(8) HUNTER, F. T., A. F. KIP AND J. W. IRVINE. J. Pharmacol. and Exper. Therap. **76**: 207, 1942.
(9) WARREN, C. O. J. Cell. and Compar. Physiol. **19**: 193, 1942.
(10) BARKER, S. B. AND W. H. SUMMERSON. J. Biol. Chem. **138**: 535, 1941.
(11) PENROSE, L. AND J. H. QUASTEL. Biochem. J. **31**: 266, 1937.

(12) VOEGTLIN, C., S. M. ROSENTHAL AND J. M. JOHNSON. U. S. Public Health Reports 46: 339, 1931.

(13) FOSDICK, L. S., O. E. FANCHER AND J. C. CALANDRA. Science 96: 45, 1942.

(14) SUMMERSON, W. H. Fed. Proc. 2: 72, 1943.

(15) FIESER, L. F. Ann. Int. Med. 15: 648, 1941.

(16) WARREN, C. O. Cancer Research 3: 1943 (in press).

(17) MÜLLER, O. H. The polarographic method of analysis. J. Chem. Education. Easton, Pa. 1941.

(18) CLARK, W. M., B. COHEN AND H. D. GIBBS. Public Health Reports 40: 1131, 1925.

(19) HARROP, G. A. AND E. S. G. BARRON. J. Exper. Med. 48: 207, 1928.

(20) DE MEIO, R. H., M. KISSIN AND E. S. G. BARRON. J. Biol. Chem. 107: 579, 1934.

(21) BALL, E. J. Biol. Chem. 106: 515, 1934.

AUGMENTATION OF BLOOD FLOW IN THE CORONARY ARTERIES WITH ELEVATION OF RIGHT VENTRICULAR PRESSURE[1,2]

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The ability of the heart to maintain its normal metabolism and work output must depend upon the capacity of the coronary vessels to supply oxygenated blood to the myocardium. Similarly, the ability of the heart to *increase* its metabolism in the presence of an *augmented load* would appear to reside in the capacity of the coronary vessels to *maintain* or even *increase* the supply of blood to the heart muscle. However, investigations in the past have failed to offer any support for this reasonable expectation. Anrep and associates (1) and Katz, Jochim and Weinstein (2), using heart-lung and isolated heart preparations in which the coronary arteries were perfused under constant pressure, found that increasing the load of either ventricle by elevation of intraventricular pressure caused a decreased flow in its corresponding coronary artery. Visscher and associates, using a special heart-lung preparation, have presented evidence that elevation of pulmonary artery pressure to the extent that the aortic-pulmonary artery pressure difference is less than 60 mm. Hg (3) or less than 40 mm. Hg (4), causes a diminution in extra-coronary sinus drainage into the chambers of the right heart and therefore presumably a diminution in coronary inflow, particularly that of the right coronary artery. Finally, Gregg (5) has reported that the differential pressure for right coronary inflow is decreased (indicating greater peripheral coronary pressure and presumably a smaller rate of flow) throughout the cardiac cycle when the pulmonary artery is partially occluded in the open chest anesthetized dog.

Even though it would appear that the heart progressively deprives itself of its own blood supply as the load upon it increases, the situation must nevertheless be regarded as one of poor economy. It would be more reasonable to anticipate that, unless the load were excessively great, an organ as vital as the heart would have at its command compensatory means by which its blood supply could be, at least in part, adjusted to its metabolic requirements.

With the introduction of the rotameter into physiological experimentation (6) a method was made available by which blood flow measurements could be made with comparative ease in many different vessels of the anesthetized dog without the necessity of seriously disturbing the "intact" state of the experimental animal. Most of the previous investigations have involved a rather extensive artificial substitution for, or supplementation to, the normal cardiovascular

dynamic circuit. The present study is an attempt to reveal the relationship of coronary flow to changes in load put upon the heart in a preparation which retains a closer approximation to the normal anesthetized dog.

PROCEDURE AND METHODS. The present investigation is restricted to the study of changes in right and left coronary inflow in the presence of an elevated right ventricular pressure. Following pre-operative morphine administration dogs were anesthetized with sodium pentobarbital. Artificial respiration was instituted, portions of the 3rd, 4th and 5th left ribs were resected, and the pulmonary and right coronary arteries exposed. Following the injection of anticoagulants (a combination of heparin, 100 U/kilo, and pontamine fast pink, 150 mgm./kilo) a trocar was inserted into the right common carotid artery to the level of the aortic valves and through this blood was led to a rotameter of appropriate design (6). For determination of changes in right coronary inflow the blood from the rotameter was led through rubber or lead tubing to the cannulated peripheral end of the right coronary artery, the central end having been tied about 6–8 mm. from its origin in the aorta. The height of the rotameter float was visually noted, and the mean rate of flow into the artery determined from the proper calibration curve. For determination of changes in left coronary inflow the procedure was essentially the same except that either the ramus descendens or left coronary artery was cannulated. In the latter instance the left coronary artery was isolated adjacent to the aorta and cannulated via a special cannula inserted into an artificial opening made in the brachio-cephalic artery. Aortic and right ventricular pressures were recorded with Gregg optical manometers (7, 8, 9). Right ventricular pressure (RVP) was elevated by an adjustable "snare" loop of cord which encircled the pulmonary artery just distal to the conus. Aortic pressure could be altered as desired by an adjustable aortic clamp applied a few centimeters above the diaphragm. In some instances the chest plate was removed. The trend of the results obtained following this more extensive exposure did not differ detectably from that obtained following only partial resection of the chest wall and will not be considered separately.

RESULTS. *Right coronary inflow versus augmentation of right ventricular pressure.* The change in right coronary inflow, in the presence of an elevated right ventricular pressure, was determined under three different circulatory conditions: 1, the pulmonary artery was gradually compressed and no effort was made to compensate any accompanying fall of aortic pressure; 2, right ventricular pressure was gradually elevated but the aortic pressure was restored to its control level by partial occlusion of the lower thoracic aorta; 3, a pre-determined and considerable amount of pulmonary constriction was abruptly applied and the fall in aortic pressure immediately compensated.

Representative data compiled from different experiments are presented in figure 1. A common ordinate scale denotes systolic and diastolic aortic (coronary perfusing) pressure, systolic and diastolic RVP, and mean rate of right coronary inflow, while the time and duration of pulmonary constriction and release are indicated on the abscissa.

Figure 1, part *A*, illustrates condition 1 above. Within 0.2 minute after pulmonary constriction was begun, right coronary inflow increased slightly from
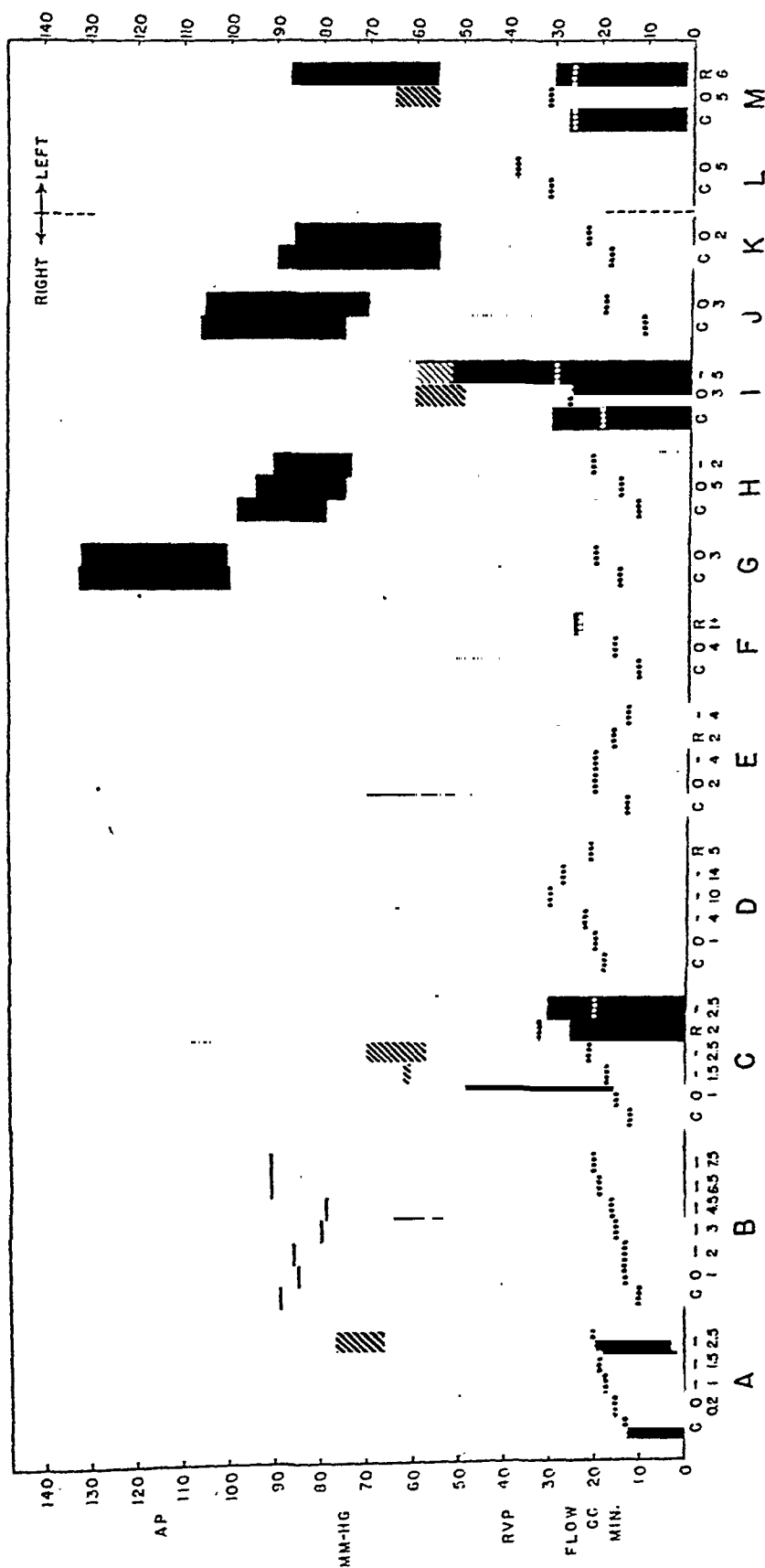
Fig. 1. Bar graphs showing the effect of augmentation of right ventricular pressure on coronary inflow (parts A through K, right coronary inflow; parts L, M, ramus descendens inflow).

Part A, right ventricular pressure gradually and progressively raised without compensation of accompanying reduction in aortic pressure. Part B, same as A but aortic pressure abruptly elevated and aortic pressure immediately compensated. Part I, right ventricular pressure eventually compensated. Abscissa—C, control; O, partial occlusion of pulmonary artery; R, release of pulmonary constriction. Numbers denote time elapsed in minutes following pulmonary artery constriction or release. Common ordinate scale denotes all pressures in millimeters of mercury and flow in cubic centimeters per minute. Lower solid bars—systolic and diastolic right ventricular pressures. Upper solid bars, systolic and diastolic aortic pressures (except for part B in which mean pressure is indicated). Dotted lines in lower bars denote values of right or left coronary inflow. Details in text.

13 to 15 cc./min. as systolic RVP rose from 30 mm. to 40 mm. Hg. Further elevation of RVP to 50 mm. Hg increased the inflow to 17.5 cc./min. while aortic pressure dropped slightly (118/81 mm. Hg). Finally the RVP was increased to 75/3 mm. Hg at which point aortic pressure fell to 100/66 mm. Hg. Despite the fall in coronary perfusing pressure, right coronary inflow continued to rise to 20 cc./min.

Part *B*, figure 1, illustrates condition 2 above in which, after a gradual and moderate elevation of RVP had progressed to the point at which aortic pressure became reduced (but right coronary inflow was still increased), the aortic pressure was compensated to its control level by the thoracic aortic clamp. This was accompanied by a further rise in RVP and an additional increase in right coronary inflow.

Part *I*, figure 1, illustrating condition 3 also shows a large increase in right coronary inflow.

The essential findings may be summarized as follows: 1, right coronary inflow begins to increase almost immediately with mild augmentation of RVP and increases progressively as systolic RVP rises to quite high levels (60–80 mm. Hg); 2, the increase in coronary inflow persists as long as RVP remains elevated, at least up to 25 minutes. (No continuous observations were made for periods longer than 25 min.); 3, the increase in right coronary inflow may occur in spite of an uncompensated reduction in aortic blood pressure[3]; 4, following release of the pulmonary constriction and return of RVP to the control values the right coronary inflow may return to the control level rather promptly, gradually, or may undergo a further but temporary increase before gradually subsiding; 5, in all dogs (31 in number) in which the aortic pressure was maintained at or slightly less than the control level, right coronary inflow increased from 25–200 per cent with pulmonary constriction, the usual increment being about 50–75 per cent of the control value.

Left coronary inflow versus elevation of right ventricular pressure. The anatomical studies of Moore (10) have indicated that in the dog's heart the ramus descendens of the left coronary artery contributes to the blood supply of the right ventricular myocardium, near its apex, along the intraventricular sulcus, and in the region of the pulmonary conus. Physiologically, it has been demonstrated that a portion of the left coronary inflow normally drains through the anterior cardiac veins over the surface of the right heart (11). Elevation of RVP might therefore be expected to increase the inflow to those portions of the right myocardium supplied by the left coronary artery. Accordingly, the inflow in the ramus descendens branch or in the left coronary artery was determined before and during pulmonary artery constriction. In all of five determinations moderate flow increases ranging from 19 to 29 per cent were observed. (For examples, c.f. fig. 1, parts *L*, *M*). Expressed in absolute amounts the flow increments compare in magnitude with those found in right coronary inflow.

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[3] Obviously, if pulmonary constriction is so severe that aortic pressure falls to a low level, the coronary perfusing pressure head will be depressed by such an amount as to cause a decrease in the coronary inflow.

DISCUSSION. In the anesthetized open chest dog, elevation of RVP (10–400 per cent) causes a significant increase in blood flow through the right coronary artery and a smaller increase in left coronary inflow. These findings are diametrically opposed to those reported by Gregg (5), using the intact dog,[4] and by Katz (2) and Visscher (3, 4), using the isolated heart, heart-lung preparation or its modification. However, although no attempt has been made in this laboratory to evaluate the adequacy of these latter preparations, it is suggested that the difference between the findings presented here and those of previous workers could well be attributed to the relative viability and physiological normalcy of the respective preparations. In the experiments presented here the animal's cardiovascular system was left intact except for the insertion of a rotameter between the common carotid or brachiocephalic artery and the coronary artery under study. The animal's own flow and pressure regulating mechanisms were undisturbed except in those instances when partial occlusion of the lower thoracic aorta was desired for the regulation of coronary perfusing pressure. Further, the elapsed time between anesthetization of the dog and the procurement of experimental data could be held in most experiments to a period not exceeding one hour.

Under these conditions the right heart of the anesthetized dog responds to an augmented load with an increase in the rate of coronary inflow. However, it should not be concluded that the increase in flow necessarily compensates fully for the increased metabolic requirements of the heart. If and when the right heart ultimately fails as the result of the increased load it will have failed in spite of an early augmentation in coronary inflow and not because the coronary inflow was initially reduced.

The cause for the observed increase in right coronary inflow and its ultimate destination in the venous channels draining the heart are being investigated.

SUMMARY

The effect of pulmonary artery constriction upon coronary inflow has been studied in the anesthetized open chest dog. Progressive elevation of right ventricular pressure by this means (to 80 mm. Hg systolic) is accompanied by a progressive and considerable augmentation in right coronary inflow (25–200 per cent) and a smaller but definite increase in left coronary inflow (19 to 29 per cent) when aortic perfusing pressure remains or is kept at the control value. Right coronary inflow may also increase in spite of a moderate, uncompensated reduction in coronary perfusing pressure (aortic pressure).

These findings, obtained in the anesthetized, open chest dog, are diametrically opposed to those reported by other investigators using different methods and preparations.

[4] The utilization of peripheral coronary pressure measurement is not now regarded by the present authors as a valid method for the determination of coronary flow. This viewpoint will be elaborated in a subsequent communication.

REFERENCES

(1) ANREP, G. V. AND H. HAÜSLER.   J. Physiol. **65**: 35S, 1928.
(2) KATZ, L. N., K. JOCHIM AND A.BOHNING.   This Journal **122**: 236, 1938.
(3) VISSCHER, M. B.   J. A. M. A. **113**: 987, 1939.
(4) MOE, G. K. AND M. B. VISSCHER.   Blood, heart and circulation.   Pub. no. 13, A. A. A. S., 1939, p. 100.
(5) GREGG, D. E.   This Journal **119**: 580, 1937.
(6) GREGG, D. E., R. E. SHIPLEY, R. W. ECKSTEIN, A. ROTTA AND J. T. WEARN.   Proc. Soc. Exper. Biol. and Med. **49**: 267, 1942.
(7) GREGG, D. E., R. W. ECKSTEIN AND M. FINEBERG.   This Journal **118**: 399, 1937.
(8) GREGG, D. E. AND D. DEWALD.   This Journal **124**: 457, 1938.
(9) GREGG, D. E. AND H. D. GREEN.   This Journal **130**: 114, 1940.
(10) MOORE, R. A.   Am. Heart J. **5**: 743, 1930.
(11) GREGG, D. E., R. E. SHIPLEY AND G. BIDDER.   This Journal **139**: 732, 1943.

THE ANTERIOR CARDIAC VEINS. THEIR FUNCTIONAL IMPORTANCE IN THE VENOUS DRAINAGE OF THE RIGHT HEART[1]

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Anatomical studies of the coronary vascular system (1) have demonstrated the presence of two separate pathways for venous drainage of the myocardium: 1, the superficial or subepicardial venous system which empties into the right atrium and consists of the anterior cardiac veins and the coronary sinus together with its contributing veins; 2, the deeper system of veins which communicates directly with the chambers of the heart. The functional significance of some of these pathways has been the subject for extensive physiological study by many investigators. The early work of Morawitz and Zahn (2) led to the belief that essentially all coronary blood was drained by way of the coronary sinus. The importance of the Thebesian or deep drainage channels was suggested by the work of Evans and Starling (3), and Markwalder and Starling (4), who showed that a very considerable portion of coronary blood drains other than through the coronary sinus. Subsequent investigators have offered abundant confirmation of the latter finding and have further indicated that the left ventricular myocardium is drained largely by way of the coronary sinus, and the right heart almost entirely by the Thebesian vessels (5, 6, 7, 8).

While these and other investigators have studied the flow from both the superficial and deep venous pathways, no consideration has been given to the possible rôle played by the anterior cardiac veins (anatomically the lesser part of the superficial cardiac venous system). The anterior cardiac veins (AC veins) are several good sized venous trunks which lie buried in the subepicardial fat occupying the sulcus between the right atrium and the right ventricle. Each is formed by the confluence of many smaller veins which course over the surface of the right ventricle and empty into the right atrium about 4 to 8 mm. superior to the ventricular edge of the tricuspid valve. Since they appeared large enough to carry a considerable volume of blood, experiments were designed to determine their functional importance as a *discrete* and *separate* exit channel for coronary blood and to estimate the contribution which the right and left coronary arteries make to the AC venous flow.

METHODS. Each experiment was performed on a dog to which sodium pentobarbital (20 mgm./kilo) was administered intravenously. (Morphine was given subcutaneously about 30 min. preoperatively.) After artificial respiration was instituted the chest plate was removed. Dissection in the subepicardial fat occupying the sulcus between the right atrium and right ventricle was carried

out starting from the root of the pulmonary artery and proceeding to the point of entrance of the coronary sinus so that each AC vein was isolated and traced to its point of entry into the right atrium. After the intravenous injection of anticoagulants (heparin—100 units per kilo and pontamine fast pink—150 mgm./kilo) small glass cannulae were inserted into the right atrium through small puncture holes made 1–2 cm. superior to the point of entry of the respective AC veins, and then each was inserted into the orifice of a vein

Fig. 1. Photograph of dog's heart showing distribution of anterior cardiac veins over the surface of the right ventricle.

and tied in place. In each dog several of the AC veins were quite small and their cannulation was not attempted. Occasionally two or three large branches of an AC vein emptied through a common atrial opening, in which case cannulation of each separate branch was made through the common opening when feasible. In several dogs, of the four or five major anterior veins present, only one, two, or three were cannulated, the remaining veins being left undisturbed.

For the measurement of AC vein flow one of two methods was employed: 1. Blood was led from each cannula through a rubber tube to a common conduit attached to a rotameter (9) from which it returned to the right auricle by way of a cannula tied into the right auricular appendage. 2. AC vein flow from the common conduit was measured in a graduate after passing through a rubber tube, the opening of which was held at a level 1–2 cm. above the level of the maximum pressure in the right atrium (as determined by the height of the blood in a vertical glass tube inserted into the right auricular appendage).

For the measurement of right coronary inflow a cannula of a length sufficient to reach to within 1–2 cm. of the aortic valves was inserted into the left common carotid artery. From the cannula blood was led through a rotameter to the peripheral end of the right coronary artery (which had been severed about 6–8 mm. from its aortic orifice and its central end tied). In many experiments a loose ligature was placed around the left coronary artery at its aortic origin for temporary occlusion of the artery when desired. Mean blood pressure was determined by means of a mercury manometer connected to the carotid cannula.

To determine the source of the blood flowing from the AC veins right coronary inflow and AC vein outflow were measured simultaneously and changes in the latter observed during a brief period when either the left coronary, the right coronary, or both right and left coronary arteries were clamped.

Sketches and/or photographs were made of all hearts showing the number and placement of the AC veins. In some hearts the major AC veins which had been cannulated were injected with a barium sulphate-gelatin mixture and roentgenograms taken.

RESULTS. *Distribution of anterior cardiac veins.* Figure 1 is a photograph showing the widespread, superficial distribution of the AC veins over the anterior surface of the undisturbed heart. Figure 2A is a roentgenogram illustrating the extensive distribution of the branches of the four major AC veins cannulated in another heart. The heart illustrated in figure 2B had three major AC veins of which one (with three branches) has been injected.[2] As observed in this and most other hearts the contrast medium was seen to pass through anastomotic channels to branches of the great cardiac vein. Several communicating veins are shown in figure 1 and figure 2B. Figure 2C is a photograph of the interior of the right atrium of another heart showing four major AC vein openings in which plugs of injection material remained after removal of the cannulae.

Of 18 hearts studied, three possessed two major AC veins, eight had three, five had four, and two had five veins. In each heart one AC vein was found which, in addition to draining a portion of the base of the right ventricle, also received sizable branches from the pulmonary conus and adjacent fat tissue (cf. figs. 2A and 2C). In all hearts a variable number of small AC veins were present which were not cannulated.

Volume of anterior cardiac vein flow. In table 1A are shown typical values for AC vein flow in dogs of different weights and with different aortic blood

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[2] The cannulae have been removed from the common opening through which the individual branches were injected.

pressures. In some experiments all of the major AC veins were cannulated; in others the flow in only a part of the total number of larger veins was deter-

Fig. 2A. (Upper left) Roentgenogram of heart in which the four major anterior cardiac veins have been cannulated and injected with contrast medium. Anterior view.

Fig. 2B. (Upper right) Roentgenogram of heart in which three branches of one anterior cardiac vein have been injected. Anterior view. See discussion in text.

Fig. 2C. (Lower) Photograph of interior of right atrium showing the position of four major anterior cardiac vein openings with plugs of injection material remaining after removal of cannulae. The arrow points to the opening of the vein which drains the pulmonary conus region (see text).

mined. In no instance were attempts made to cannulate the small veins. In those experiments in which all of the major AC veins were cannulated, the flow

values range from 8.5 to 26.5 cc. per minute. The flow from the AC veins may be augmented by clamping the thoracic aorta or the injection of epinephrine. The effects of the latter two procedures are illustrated in table 1, part B.

Source of anterior cardiac vein flow. To establish the source from which the AC vein flow arises, the right and left coronary arteries were both occluded for 25–30 seconds. The blood pressure fell not more than 5–10 mm. Hg during

TABLE 1

DOG WEIGHT	MEAN BP	FLOW		RATIO (%) AC FLOW (3) ÷ RT. COR. FLOW (4)	REMARKS
		Anterior cardiac veins	Right coronary artery		
1	2	3	4		
Part A—Normals					
kgm.	*mm. Hg*	*cc./min.*	*cc./min.*		
21.3	85	12.0	12.0	100	Flow measured in all 3 major AC veins
24.0	90	19.0	21.5	88	Flow measured in all 3 major AC veins
24.0	120	26.5	37.0	72	Flow measured in all 3 major AC veins
19.3	94	16.5	15.0	110	Flow measured in both major AC veins
18.2	82	13.5	26.0	52	Flow measured in 2 of 4 major AC veins
21.5	70	12.0	12.0	100	Flow measured in 3 of 5 major AC veins
Part B—Variables					
18.3	60	26.0	25.0	104	*Control*—Flow measured in all 5 major AC veins
	86	34.0	30.0	113	Constriction of aorta
16.5	70	5.0	6.8	74	*Control*—Flow measured in 1 of 2 major AC veins
	125	10.8	11.0	98	Constriction of aorta
19.3	70	12.0	12.0	100	*Control*—Flow measured in both major AC veins
	105	30.0	25.5	118	Epinephrine by jugular vein
16.5	70	5.0	7.0	71	*Control*—Flow measured in 1 of 2 major AC veins
	125	21.0	18.0	117	Epinephrine by jugular vein
	125	35.0	33.0	106	Epinephrine by jugular vein
21.3	120	6.0	8.0	75	*Control*—Flow measured in all 3 major AC veins
	110	13.0	20.0	65	Nitroglycerine by jugular vein

this period. In one experiment the flow from the AC veins was reduced almost to zero. In other experiments a residual flow remained (0.5–1.0 cc.) equal to 5–10 per cent of the control AC flow. Examination of the latter hearts at the close of the experiments revealed the presence of 1 or 2 right coronary artery twigs central to the right coronary cannula which were not occluded during the experimental procedure. Therefore, almost all if not the entire anterior vein flow must be regarded as having its origin in the coronary arteries.

Since the AC veins are anatomically disposed over the surface of the right ventricle, it would seem probable that they drain a myocardial area which is largely supplied by the right coronary artery. Simultaneous measurements of right coronary inflow and AC vein outflow (all major veins cannulated) show in different experiments that the latter may vary from 72 to 118 per cent of the former and that both vary in the same direction under the influence of altered dynamic conditions (cf. table 1, A, B).

The AC vein blood arises from both coronary arteries (cf. graphs of fig. 3 for typical data). Temporary clamping of the left coronary artery causes a slight rise in right coronary inflow while the AC vein outflow diminishes. Similarly, when the right coronary artery is clamped, the AC vein flow again diminishes, but a considerable residual flow (arising from the left coronary artery) remains. As established by such clamping procedures, the respective contributions of the two coronary arteries to the AC vein flow are variable in different experiments, but the major contribution is from the right coronary artery.

DISCUSSION. Measurements of outflow from the AC veins in the open chest, anesthetized dog have demonstrated the functional significance of these vessels in draining the myocardium, particularly that of the right ventricle. It follows, therefore, that any consideration of the venous return from a coronary bed must include recognition of the rôle played by the AC veins as well as that of the coronary sinus, Thebesian, and other luminal vessels.

It is significant that blood leaving via the AC veins is almost, if not entirely, derived from the coronary arteries. In view of the anatomical disposition of the AC venous system over the right ventricle, it is not unreasonable to expect that AC vein blood would originate largely in the right coronary artery, nor is it difficult to imagine that a considerable portion of right coronary blood could be drained by this anatomically intimate and relatively extensive system of venous channels. On the same basis, the appearance of left coronary blood in the AC veins might have been anticipated. Inspection of the surface of the heart reveals that small twigs of the left descending branch supply a "fringe" of the septal border of the right ventricle and pulmonary conus from which many of the fine branches of the AC veins may be seen to arise. In the same area branches of the great cardiac vein may be seen to anastomose with those of the AC veins (cf. fig. 1 and fig. 2B).

It is evident that the total AC vein flow recovered from the major veins cannulated originates predominantly in right coronary artery although the relative amount varies in different dogs. The individual AC veins also vary with respect to the amount of blood contributed to each of them by the two coronary arteries. Although most of the veins carry predominantly right coronary blood, the AC vein which drains the pulmonary conus region (figs. 2A and 2C) derives its blood in greater part from the left coronary artery.

On the basis of the data already presented in figure 3 an approximation can be made of the amount of right coronary inflow which is drained via the anterior cardiac veins. In different experiments from 50–92 per cent of right coronary
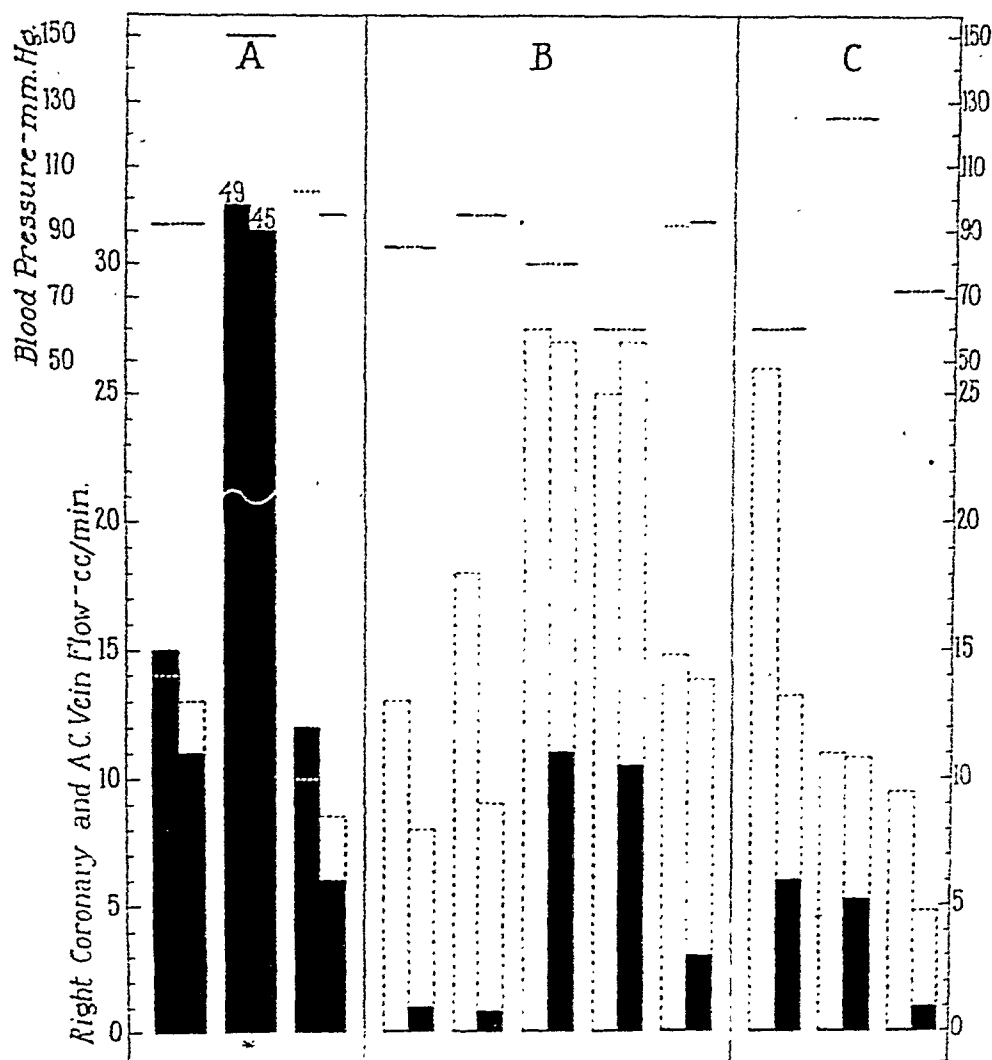
Fig. 3. Chart of data from different experiments illustrating: 1, the relative volumes of flow measured simultaneously in the right coronary artery and anterior cardiac veins; and 2, the source of the anterior cardiac vein flow as shown by clamping of either the left or right coronary artery. In each part the bar on the left represents the rate of right coronary inflow, and that on the right, the rate of anterior cardiac vein outflow. Dotted lines (and areas) in each pair indicate the control values for both arterial and venous flows. Solid lines (and areas) represent the flow values after clamping of a coronary artery as indicated below. Part A. Flow in right coronary artery versus flow from all major anterior cardiac veins before and during occlusion of left coronary artery. *Epinephrine given intravenously and no control flows obtained before occlusion of left coronary artery. Part B. Flow in right coronary artery versus that from all major anterior cardiac veins before and during occlusion of right coronary artery. Part C. Same as for part B except that anterior cardiac vein flow was measured from 2 out of 4, 1 out of 2, and 1 out of 4 major AC veins, respectively. Transverse dotted lines and bars in upper part of chart, mean aortic blood pressure. Flow (cc. per min.) in right coronary artery and anterior cardiac veins and mean aortic blood pressure (mm. Hg) indicated on ordinate scales.

inflow is found to drain into the right atrium by way of the AC veins. These values were derived both directly and indirectly. The measurement of right

coronary inflow and AC vein outflow during a temporary complete occlusion of the left coronary artery allowed direct comparison of the two recorded flows without interference from blood entering via the left coronary. Indirectly, right coronary drainage through the AC veins can be estimated by observing the amount by which the AC vein flow diminishes when the right coronary artery is temporarily occluded. As mentioned above, these two methods probably give only approximate percentages for the relative amount of right coronary blood draining via the AC veins. However, it is suggested that not only the values of 50–92 per cent but also those for the total AC vein flow probably err on the conservative side for one or more of the following reasons: 1. Clamping of the left coronary artery will markedly reduce coronary sinus outflow, thereby establishing a favorable pressure gradient for an increase in the non-AC vein drainage of the right ventricle. 2. Similarly, clamping of the right coronary artery initially reduces the AC vein outflow (by an amount equal to the artery's original contribution before clamping) which in turn affords an easier pathway for blood from the left coronary artery to leave the right myocardium by way of the AC veins. Consequently the recorded residual AC vein flow which remains after clamping of the right coronary artery may exceed that which is normally drained from the left coronary artery, and the right coronary contribution, found by subtraction of the residual flow from the original flow, therefore appears to be less. 3. The manipulation and transient occlusions of the AC veins incident to their isolation and cannulation could conceivably promote collateral flow from the AC veins through anastomotic channels (c.f. figs. 1 and 2B) to the coronary sinus. This possibility is supported by the observation that flow from the AC veins may be permanently diminished (for the duration of the experiment) by clamping them for 1–2 minutes. 4. The resistance to outflow imposed by the small AC vein cannulae, rubber tubes (and rotameter) and any unrecognized torsion or flexion of the several cannula tips in their veins must cause a greater limitation of flow than that which exists in the uncannulated veins emptying directly into the right atrium. 5. In all experiments only major AC veins were cannulated and several small to medium sized veins were always present from which the flow of blood was not measured.

Consideration of the above factors offers reasonable justification for the belief that, in the undisturbed heart, the AC veins normally drain not only a greater absolute quantity of blood but also a greater percentage of the right coronary inflow than even the experimentally determined values of 50–92 per cent.

The general interpretation of the findings of Katz, Jochim and Weinstein (6) is that approximately 90 per cent or more of right coronary inflow drains via the Thebesian channels of the right heart. The present study reveals that the AC veins (and not the Thebesians) constitute the major drainage pathway for the right coronary artery. While the findings presented here would appear to be entirely incompatible with those of Katz and associates, careful reading of their communication reveals that the authors have measured total flow issuing from the chambers of the right heart and refer to it as arising from both "acces-

sory veins and Thebesian channels." On the basis of the results presented here, it is tempting to conclude that this combined flow in their experiments originates in large part from the AC veins. However, because of the failure of these authors to separate Thebesian and AC vein flow, such a conclusion is not justified; on the other hand, it is equally fallacious to assume *that the combined flow in their preparation is entirely or even predominantly Thebesian.*

The apparent reluctance of many investigators to consider the possible significance of the AC vein flow (even when it is measured together with Thebesian flow) would seem to reflect either a specific favoritism for the rôle of the Thebesian vessels, or an unwarranted, yet conventional neglect of a known, but heretofore virtually ignored, venous drainage system.

It is suggested that all studies of cardiac venous channels, flow partitions, and Thebesian drainage, which have been made in the past without consideration of the rôle of the anterior cardiac veins, should be subjected to a critical re-evaluation before the associated conclusions can be accepted as being valid.

SUMMARY

The number and anatomical distribution of the major anterior cardiac veins of the dog's heart have been studied from roentgenograms of specimens injected immediately postmortem. Inspection alone reveals that the greater portion of the subepicardial surface of the right ventricle is traversed by many small branches which merge to form, in different hearts, from two to five major anterior cardiac veins. Each major vein empties separately and directly into the right atrium about 4 to 8 mm. superior to the border of the tricuspid valve. Other smaller veins are invariably present.

The functional importance of the anterior cardiac veins, virtually ignored by previous investigators, is demonstrated. In 16 anesthetized open chest dogs with different body weights and blood pressures, the flow from all the major anterior cardiac veins cannulated ranged from 8.5 to 26.5 cc. per minute. The flow could be increased greatly by various procedures. It was established that flow from the anterior cardiac veins is almost, if not entirely derived from the coronary arteries, and its magnitude generally approaches and not infrequently exceeds the simultaneously measured right coronary inflow.

The major portion (50–92 per cent) of right coronary inflow was found to drain via the anterior cardiac veins into the right atrium. This finding makes completely untenable the conventionally accepted belief that nearly all of the right coronary inflow drains by way of the Thebesian vessels into the right ventricle.

To prevent confusion in terminology for the cardiac veins considered here, it is suggested that the officially accepted name "anterior cardiac veins" (BR of BNA) be used when indicated, and the conventional use of the misleading term "accessory veins," as applied to an aggregate of several unspecified vessels, be avoided.

REFERENCES

(1) GRANT, J. C. B.   A method of anatomy, p. 507, 1940.   Williams & Wilkins, Baltimore.

(2) MORAWITZ, P. AND A. ZAHN.   Zentralbl. f. Physiol. 26: 465, 1912.

(3) EVANS, C. L. AND E. H. STARLING.   J. Physiol. 46: 418, 1913.

(4) MARKWALDER, J. AND E. H. STARLING.   J. Physiol. 47: 275, 1914.

(5) ANREP, G. V., A. BLALOCK AND M. HAMMOUDA.   J. Physiol. 67: 87, 1929.

(6) KATZ, L. N., J. JOCHIM AND W. WEINSTEIN.   This Journal 122: 252, 1938.

(7) MOE, G. K. AND M. B. VISSCHER.   This Journal 125: 461, 1939.

(8) MOE, G. K. AND M. B. VISSCHER.   Blood, heart and circulation.   A. A. A. S. no. 13, p. 100, 1939.

(9) GREGG, D. E., R. E. SHIPLEY, R. W. ECKSTEIN, A. ROTTA AND J. T. WEARN.   Proc. Soc. Exper. Biol. and Med. 49: 267, 1942.

THE PRESENCE IN NORMAL URINE OF CORTIN-LIKE MATERIAL WHICH IS ACTIVE IN A MUSCLE WORK TEST

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It has recently been shown that normal male urine contains extractable material which possesses certain of the physiological properties of the adrenal cortical hormones. The extract shows the following biological activities when tested in the adrenalectomized rat: 1, it increases resistance to cold (1); 2, it maintains life (2); 3, it elevates liver glycogen (3). In the present communication it will be shown that this extract is also active when assayed by a muscle work test.

Gans and Miley (4) showed that the gastrocnemius muscle of the adrenalectomized rat when stimulated to contract under load for a number of hours lost its contractility very much sooner than the muscle of the normal animal. Hartman, Brownell and Lockwood (5) and Hales, Haslerud and Ingle (6) have confirmed this observation, and have shown that adrenal cortical extract is capable of prolonging the poorly sustained contractility which ensues after adrenalectomy. Ingle (7) has shown, moreover, that progesterone, testosterone, and estradiol are inactive and that desoxycorticosterone is considerably less active than those cortical steroids with an oxygen at position 11. It would appear, therefore, that the muscle "fatigue" test should be relatively specific for adrenal cortical hormones, and particularly for those steroids of the latter category.

METHODS. The urinary extracts were prepared as has previously been described (1). The method consisted briefly of an extraction of pooled urine from normal men with ethylene dichloride and a purification by removal of inert alkali soluble material with sodium hydroxide. The final extract was taken up in 10 per cent ethanol and adjusted to a volume so that 1 cc. was equivalent to 1 liter of urine.

Male rats of the Sprague Dawley strain, weighing between 120 and 150 grams, were used. It was discovered early in the course of the experiments that only those animals which were in perfect condition and entirely free from respiratory infections were suitable for use. Dyspnea due to mucus in the respiratory tract was seen to cause marked impairment of muscle contractility. Three days after adrenalectomy the rats were given 1 cc. of the extract by stomach tube. Controls received either 1 cc. of water or 1 cc. of 10 per cent ethanol. A solution of sodium pentobarbital (2 mgm. per 100 grams of rat) mixed with sodium barbital (28 mgm. per 100 grams) was then injected intraperitoneally. Four cubic centimeters of water was injected subcutaneously just before the start of stimulation and a similar dose was administered 5 hours later.

Through a circular incision near its attachment the gastrocnemius tendon was isolated, cut, and attached to a muscle lever loaded with a 50 gram weight.

Through another small incision the tibia was immobilized in a semi-vertical position by means of a small-jawed clamp. Into the distal region of the muscle a silver wire electrode was inserted.

Stimulation was accomplished by means of a Harvard inductorium. The source of the current was a 2 volt storage battery and the interrupting device consisted of a flat weighted flexible strip of steel with a wire attached to the free end which made intermittent contact with a mercury cup. Vibration was maintained by a magnet attached above the region of the fixed end of the spring. The rate of stimulation was three per second, the stimulus was super-maximal, and both make and break shocks were used. Two rats were connected in series and in the great majority of cases one of these animals served as an untreated
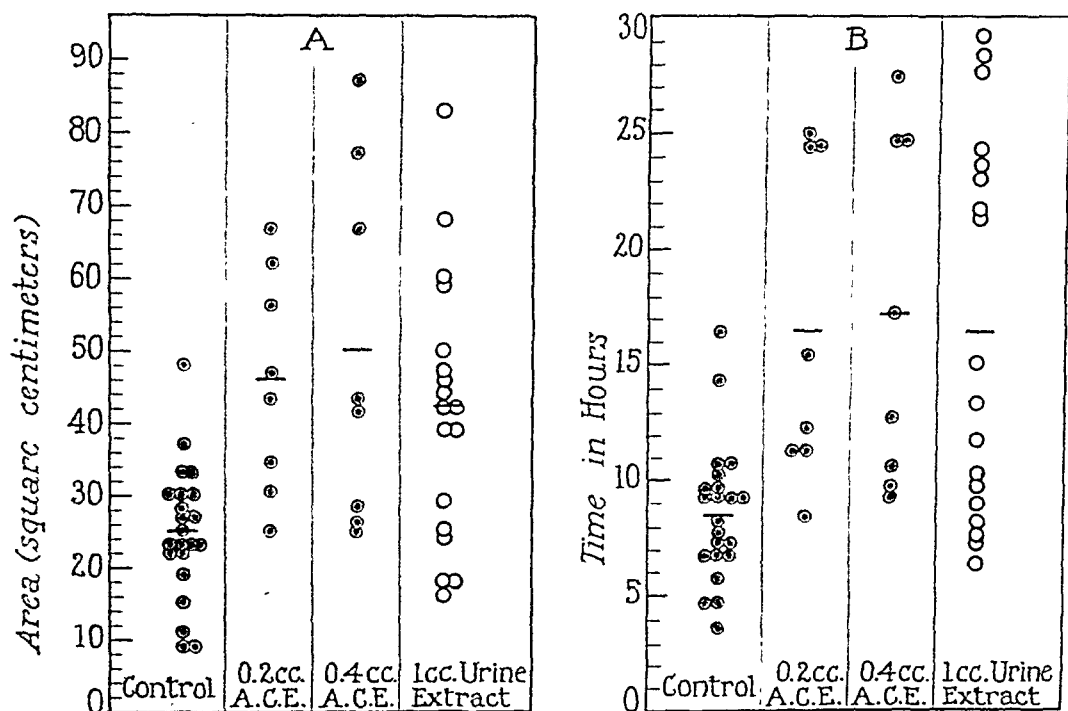
Fig. 1. Muscle performance of adrenalectomized rats after injection of urinary extract and adrenal cortical extract. The readings in part A represent work performance while those in part B represent time elapsing until contractions ceased. The horizontal lines indicate the average for each group.

control. Contractions were recorded on a smoked drum which was made to revolve at constant speed so that one revolution was completed in 30 hours. Work performance was estimated by measurement of the area of the record of contraction. The animals were enclosed in a chamber in which the temperature was held at 28°C. ± 0.5. Stimulation was not continued longer than 29 hours.

Kymographic recording of muscle contraction has one advantage over mechanical counting devices. It allows one to determine whether there is a true gradual muscle "fatigue" before the death of the animal. A gradual decline in muscle contractility was considered essential for an acceptable assay. Many rats for one reason or another die abruptly while muscle contractility is still unimpaired. All records which indicated this course of events were excluded from the present series.

RESULTS. The average performance of the rats which received the urine extract was 70 per cent greater than that of the controls (fig. 1 A). In view of the fact that the difference between the two means was 3.8 times its standard error, this increase may be considered to have definite significance.

Sham operated rats showed at the end of 30 hours a height of contraction which was only slightly less than at the beginning of stimulation. Because the true limit of performance of these rats can not be estimated without prolonging the experiments for a number of days this group was not included in the table.

Included in figure 1 are responses obtained with Wilson's adrenal cortical extract (1 cc. ≈ 75 grams of gland). Two-tenths cubic centimeter of extract produced an 80 per cent increase and 0.4 cc. a 100 per cent increase in work performance. The extract from one liter of urine gave a response of the same general magnitude as 0.2 cc. of the adrenal cortical extract.

As others have previously pointed out (6), it is unlikely that the poorly sustained muscle contractility which is observed in adrenalectomized animals is due to simple intrinsic fatigue. In the 50 animals used for assay, with only one exception, death occurred within 3 hours after failure of contraction. It would seem that muscle failure occurred because the animal was approaching death, and that the values obtained for work performance were largely a reflection of the ability of the animal to survive under the stress imposed by the conditions of the experiment. As seen from figure 1 B the time that contractility is made to endure is as good a measure of the activity of an extract as is work performance.

DISCUSSION. The same extract which was found active by the muscle work test here employed has also been shown to possess activity in the cold protection test. Furthermore, it has been shown to maintain the life of adrenalectomized rats and to elevate liver glycogen. This urinary preparation is thus seen to possess all of the physiological properties of adrenal cortical extract which have been tested for up to this time. The present experiments lend weight to the possibility that urine contains certain unidentified active steroids which have their origin in the adrenal cortex.

SUMMARY

The urine of normal men contains extractable material which is active in a muscle work test. The extract from one liter of urine gave a response roughly comparable to that produced by 0.2 cc. of Wilson's cortical extract.

REFERENCES

(1) DORFMAN, R. I., B. N. HORWITT AND W. R. FISH. Science 96: 496, 1942.
(2) DORFMAN, R. I. AND B. N. HORWITT. Federation Proceedings 2: 60, 1943.
(3) HORWITT, B. N. AND R. I. DORFMAN. Science 97: 337, 1943.
(4) GANS, H. M. AND H. H. MILEY. This Journal 82: 1, 1927.
(5) HARTMAN, F. A., K. A. BROWNELL AND J. E. LOCKWOOD. Endocrinol. 16: 521, 1932.
(6) HALES, W. M., G. M. HASLERUD AND D. J. INGLE. This Journal 112: 65, 1935.
(7) INGLE, D. J. Endocrinol. 26: 472, 1940.

A COMPARISON OF MOTOR INTEGRATION IN THE MOUSE, RAT, RABBIT, DOG AND HORSE[1,2]

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The automatic rhythmical recurrence of inspiratory contractions, their simplicity of pattern, their dependable uniformity and their ease of electrical registration suggested using them in a comparative study of motor integration. Five mammals, the mouse, rat, rabbit, dog and horse were chosen for their differences in size and for differences in nimbleness of movement often associated with size.

RESULTS. Contractions of the diaphragm of the mouse, rat, rabbit, dog and horse, recorded electrically with widely separated electrodes to include the activity of numerous muscle units, show characteristic electrical fusillades whose patterns of increasing density and amplitude of electrical deflections are virtually indistinguishable from one another (see figs. 4, 5, 6, 7 and 8). On the basis of older experiments on the dog the increasing density and amplitude of electrical deflections in each of these figures is interpreted to indicate a progressively increasing activity of the muscle units plus an increasing coincidence and summation of potentials of synchronously contracting units (Gesell, Atkinson and Brown, 1941). Similarity of configuration of the electrical fusillades would therefore be strong evidence in itself for a common underlying mechanism of central integration of inspiratory contractions in mammals. With that in mind, we have attempted to resolve the fusillades of each group of mammals into their component parts. This was accomplished by placing leading-off electrodes on the surface of a relatively inactive portion of muscle where few muscle units were contracting (see figs. 10-14 and 15-20). With fortunate placement such procedure allows analysis of individual activity of 1, 2, 3, or even 4 muscle units throughout the course of inspiration. By increasing the chemical stimulus to breathing, physiological modification of activity of the individual units can be observed.

Dog. Abundant observations on the respiratory contractions of the dog (see review by Gesell, 1940) offer a suitable starting point of comparison. Progressive strengthening of contraction, as inspiration advances, is characteristic (figs. 1 and 7). This effective adjustment to increasing mechanical resistance is attained by a simple arrangement of multifiber twitching consisting of a progressive recruitment of newly activated units plus a progressive quickening of twitch rhythm of the activated muscle units (see fig. 1). The schema reads from left to right. Muscle unit 1 leads the contraction, twitching with increasing frequency as inspiration progresses. Before inspiration is ended, 28 dormant units

are recruited along the left diagonal, each successive unit adding a decreasing number of twitches. As each inspiration advances more units contract in unison
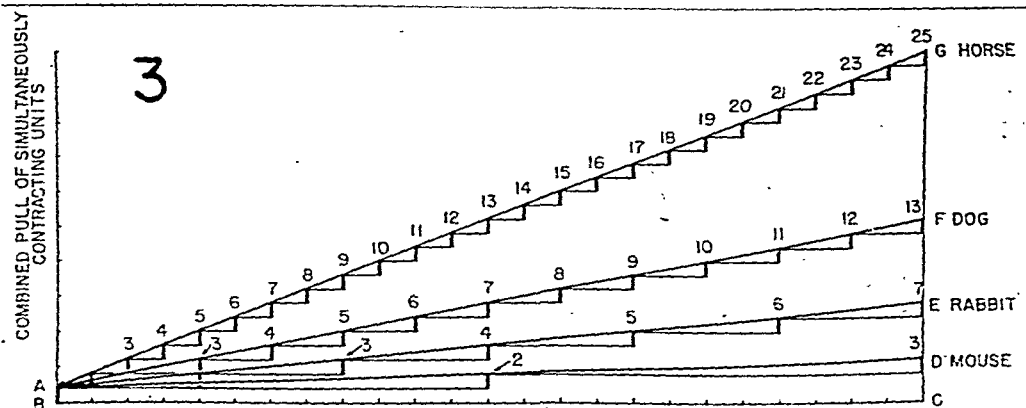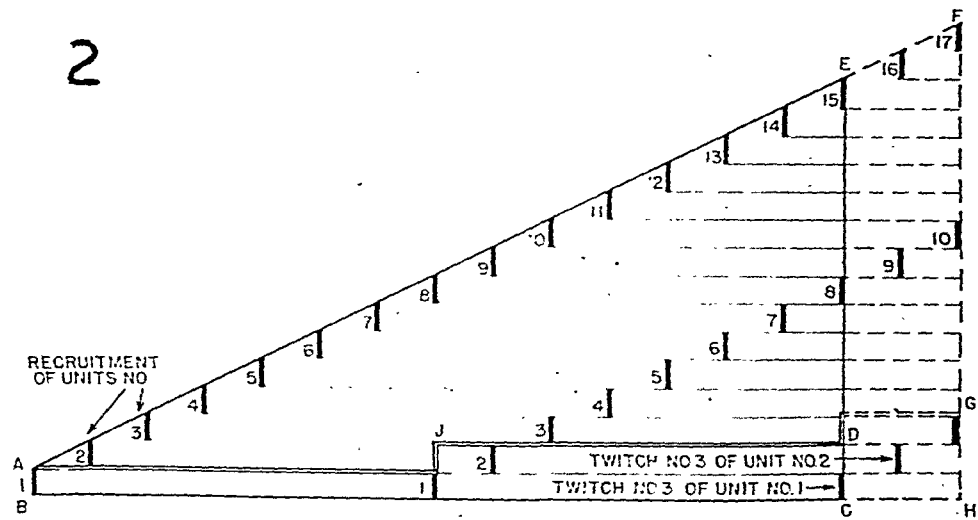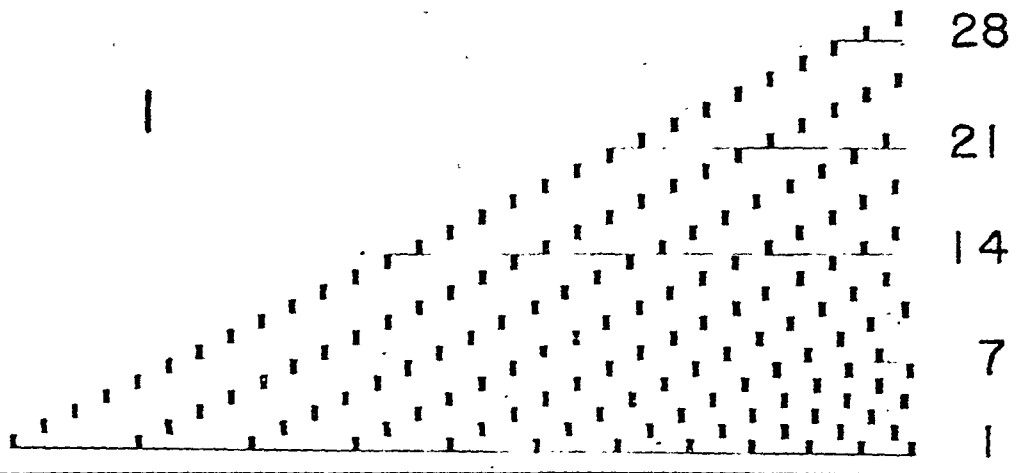
Figs. 1-3

and the sum total of their pulls increases to a relatively uniform maximum intensity. Subsidence of contraction is much faster (Gesell, Atkinson and Brown, 1941).

Rat. The rat shows the same type of recruitment and twitch frequency control in normal eupneic inspirations as those illustrated in figure 1 and also similar intensifications of these processes by increasing chemical stimulation of breathing as those observed in the dog. These facts are evidenced in progressively augmenting contractions, figures 15, 16, 17, 18, 19 and 20, in which the twitches of 3 muscle units can be followed during increasing chemical stimulation (rebreathing). In contraction 1 (fig. 15) only one muscle unit is active in the proximity of the electrodes. It twitches 11 times in all and with increasing frequency. Units 2 and 3, for the present dormant, are to be recruited as the contractions become stronger. Augmentation of contraction is evident in the increasing sum total of twitches per inspiration (11, 13, 16, 19, 24 and 39 twitches respectively). The nature of the intensification is revealed in the number of twitches of the individual units (11 + 0 + 0) (12 + 1 + 0) (13 + 3 + 0) (14 + 4 + 1) (15 + 6 + 3) (18 + 10 + 11), and the timing of these twitches in the inspiratory phase (see records).

Mouse. Recruitment and increasing frequency of muscle fiber twitch as inspiration advances and intensification of these mechanisms by asphyxia are as characteristic for the mouse as for the dog and rat. Figures 10, 11, 12, 13 and 14, arranged in order of intensity and not in order of occurrence, are of interest in showing that sporadic irregularities in depth of breathing, occurring during a continued *uniform* chemical drive, are also dependent upon orderly recruitment and twitch frequency control. The stronger the contraction the greater is the number of twitches of the newly recruited unit, the higher is the maximum twitch frequency and the earlier is the participation in contraction.

Rabbit. Figure 29, obtained from the diaphragm of the rabbit, is a rather exceptional illustration showing the precision and the nicety of control of muscular contractions. It reveals in terms of twitches of a single newly recruited unit how increasing chemical demands (rebreathing) are met by increasing mechanical response. The leading-off electrodes were resting on a very inactive portion of the muscle, as is witnessed by the faintly visible activity of a single distant unit. Fortunately this unit is active throughout each period of inspiration (eupneic as well as hyperpneic) and, therefore, automatically marks the beginning and end of each inspiration.

The first breath is eupneic and shows activity in the distant unit only. At the end of the second breath a newly activated unit contributes two twitches. This unit shows increasing activity as rebreathing continues. In the third breath it contracts 4 times instead of twice, then 6, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19 and 20 times, thus meeting the growing needs of asphyxia with appropriate liberation of mechanical energy. Note that the rhythmic activity of the newly recruited muscle unit is lowest at the beginning of each series of twitches regardless of the moment in the phase of inspiration at which activity begins, that the frequency of twitch increases as each series of twitches approaches its end, and that the twitch frequency attains a greater maximum the longer the period of activity (i.e., the more nearly complete the recruitment). These observations indicate that in the rabbit, as in the dog (fig. 1), the forces producing recruitment and acceleration of twitch increase to a maximum at the end of inspiration.
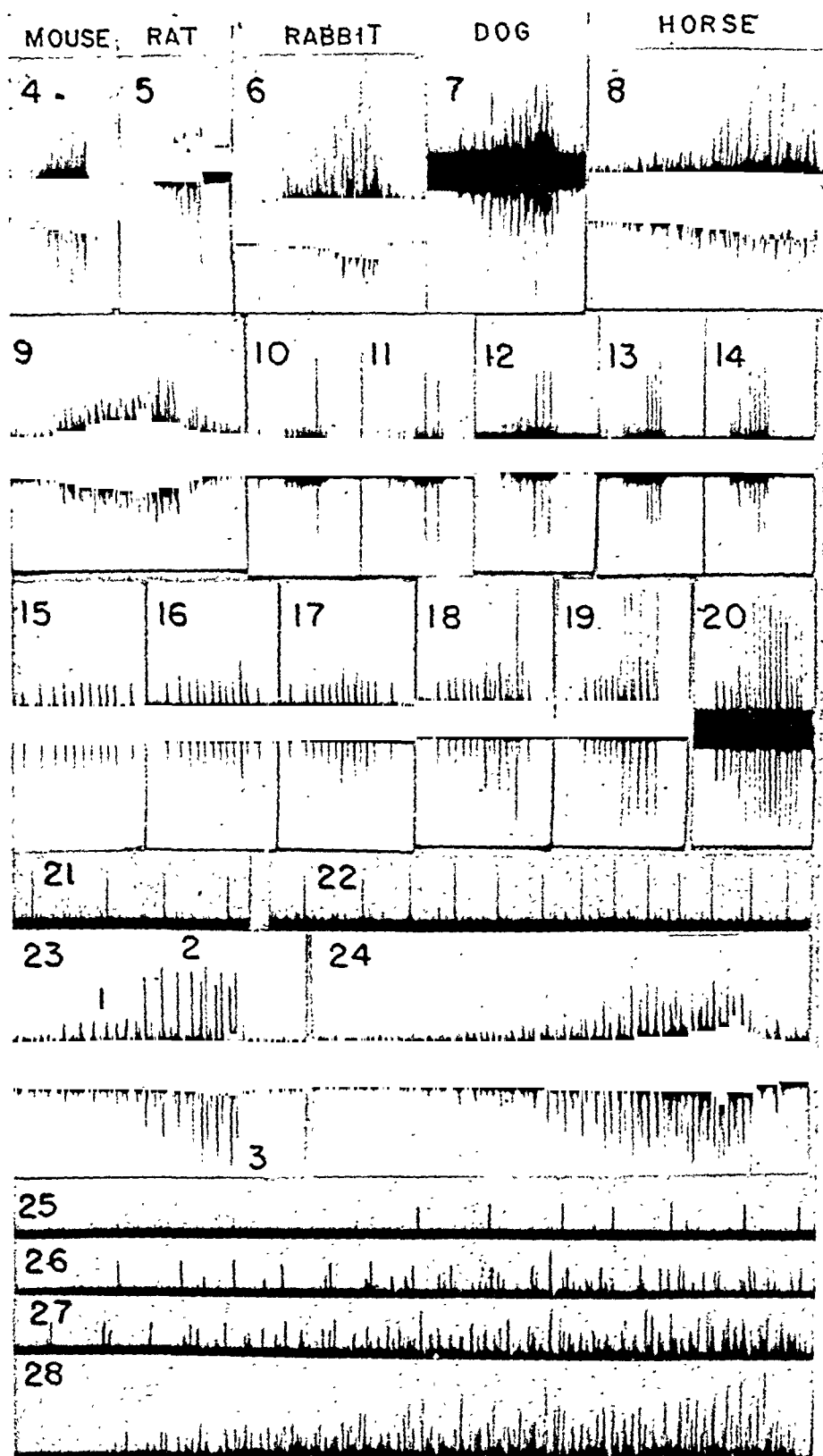
Figs. 4-28

Adrian and Bronk (1928) studying the discharge in single fiber preparations of the phrenic nerve of the rabbit could find no clear evidence for recruitment in that no single fiber was observed to be active for a period significantly shorter than the inspiratory discharge of the whole nerve. Nor did they observe increasing frequency of twitch of the individual units during the course of inspiration. Such "rectangular" contractions, beginning and ending abruptly, stand in contrast to the slowly increasing "triangular" contractions illustrated in figures 4, 5, 6, 7 and 8. To explain greater strength of diaphragmatic contractions produced by asphyxia they proposed "as far as concerns that part of the muscle supplied by the third cervical root of the phrenic" that gradation is "due
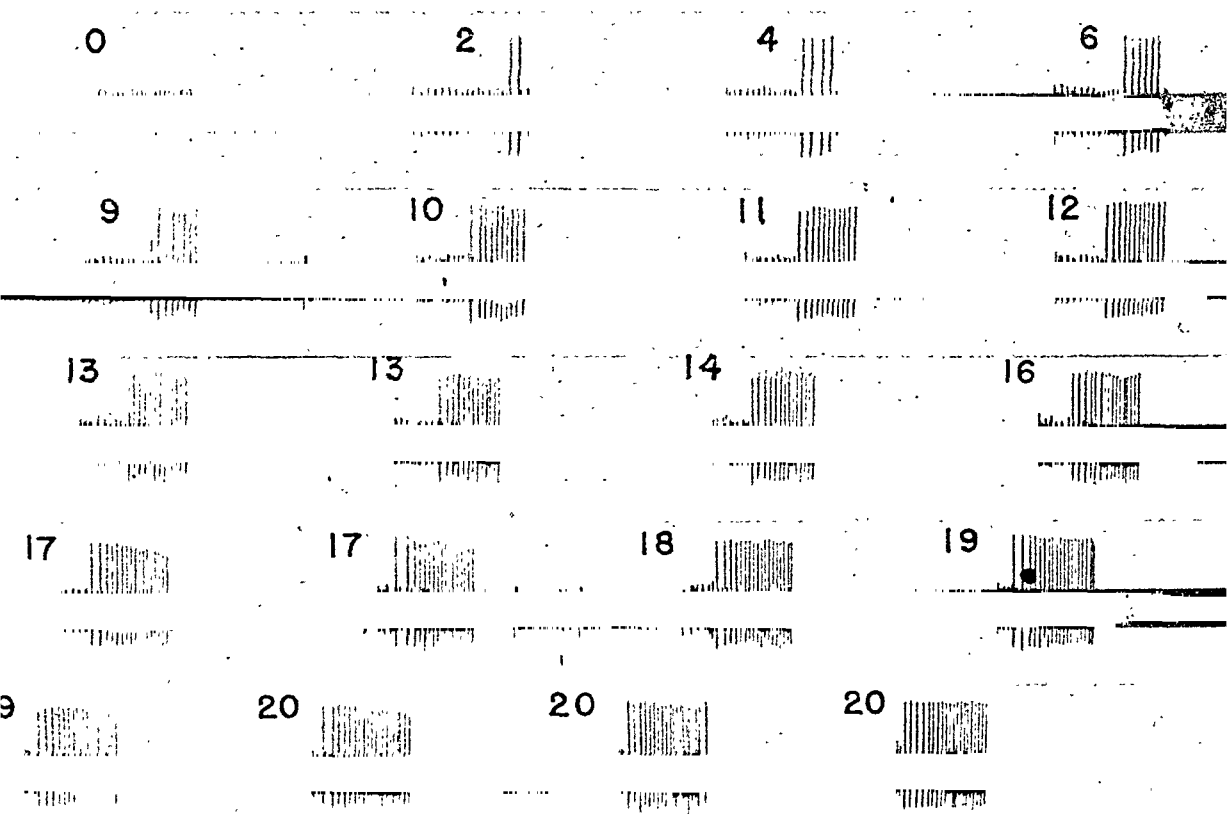
Fig. 29

mainly to the increased number of impulses which reach the muscle from each nerve fiber." Similar views were expressed for the flexion reflex. In the extension reflex and in the inspiratory contractions of the intercostal muscles the number of active fibers and their frequency of discharge were however found to increase as contraction progressed (Bronk and Ferguson, 1935).

Because our results were obtained from the diaphragm rather than from its motor nerve, we have made a few observations on the phrenic nerve as well. As is seen in figure 9, inspiratory fusillades of the phrenic nerve trunk of the rabbit are similar to diaphragmatic fusillades (figs. 4, 5, 6, 7 and 8). Observations on smaller fractions of the nerve reveal that recruitment and increasing

frequency of nerve cell discharge are comparable to recruitment and twitch frequency changes in muscle. Since all parts of the diaphragm of the rabbit seemed to contract in a similar fashion, it is concluded that our findings on the rabbit are essentially the same as those on the mouse, rat and dog.

Horse. The events of contraction in the horse (figs. 21 to 28) are essentially the same as those in the preceding mammals. Incompletely recruited muscle units (see fig. 21) contract late in the inspiratory phase and at a low frequency. When more nearly completely recruited (rebreathing) they contract earlier and attain a higher frequency (see fig. 22). There are 4 twitches in the first inspiration as compared with 13 in the second. Maximum twitch frequency is 2.5 per second in figure 21 as compared with 5 per second in figure 22. Recruitment of new muscle units during the course of an ordinary eupneic inspiration increases progressively in the usual orderly way (see fig. 23 showing three sets of discrete potentials). Muscle unit 1 contracts relatively early in the phase of inspiration, twitching approximately 20 times. Muscle unit 2 follows later, twitching 7 times, while unit 3 beginning still later, twitches only 3 times in all. The electrogram in figure 24 represents a considerably deeper breath, occurring sporadically in the same horse. Many more muscle units come into play. The fusillade becomes more dense, consequently concealing the discreteness of activity of individual muscle units noted early in the phase of inspiration. The following four records (figs. 25, 26, 27 and 28) are from a single setting of the leading-off electrodes on the diaphragm of another horse subjected to increasing chemical stimulation of rebreathing. The important rôle of recruitment is evident.

Other animals. Adjustment of muscular contraction to mechanical requirements such as described for mammals is undoubtedly a survival of a primitive mechanism for it is found to exist in the inspiratory contractions of the bird and reptile as well as in the mammal. It is found in expiratory contractions of insects, reptiles, birds and mammals which also meet with increasing resistance as contraction advances (Loofbourrow and Gesell, 1941).

Parallelism of respiratory and motor neuron rhythm. Under the conditions of our experiments, the horse, dog, rabbit, rat and mouse breathed at a frequency of approximately 8, 15, 50, 65 and 100 per minute respectively (see table). The corresponding durations of the inspiratory phase of eupnea were roughly 2.4, 1.3, 0.6, 0.4 and 0.3 of a second. The average eupneic twitch frequency of fully recruited muscle units at the end of inspiration, in round numbers, was about 10, 20, 50, 40 and 100 per second. These figures, derived from limited observations, are at best approximations only. Large ranges in twitch frequency extending between 5 to 16 in the horse, 10–30 in the dog, 25–60 in the rabbit, 25–45 in the rat and 60 to 150 in the mouse, give no sure indication where the average for eupnea lies. Anesthesia, magnitude of prevailing chemical drives, and the condition of individual animals probably all contribute to this great range. Nevertheless our table is suggestive on several points.

The most striking and pertinent relationship is the tendency towards parallelism between respiratory rhythm and muscle fiber twitch rhythm. The mouse breathes approximately 10 times as frequently as the horse. Its muscle fibers

twitch in the same ratio.   The rabbit, breathing 6 times as frequently, has a muscle fiber rhythm about 5 times that of the horse.   The dog, breathing about twice as often, has a muscle fiber twitch rhythm approximately twice that of the horse.   This simple relationship implies that nimbleness of body and nimbleness of individual nervous integrating units tend to go hand in hand.

The twitch frequency of the rat, compared with that of the rabbit, suggests a deviation from a strictly inverse relation to animal size.   Factors other than mass conceivably play a rôle in the evolution of nimbleness, e.g., the security of the porcupine and skunk against attack might be a reasonable explanation of their deliberate movements.   These animals were unfortunately not available for a study of their underlying motor integrations at the time of our experiments.

Parallelism of respiratory rhythm and of motor neuron and muscle fiber twitch rhythm in the mouse, rabbit, dog and horse should tend to produce approximately the same maximum number of twitches per inspiration in those units which lead off the inspiratory act.   Direct observations indicate a reasonable

TABLE 1

ANIMAL	AVERAGE NUMBER OF BREATHS PER MINUTE	APPROXIMATE AVERAGE DURATION OF THE INSPIRATORY PHASE	RANGE OF MUSCLE FIBER TWITCH FREQUENCY PER SECOND	APPROXIMATE AVERAGE NUMBER OF MUSCLE FIBER TWITCHES PER SECOND	COMPUTED NUMBER OF TWITCHES PER INSPIRATION USING OBSERVED FREQUENCY OF TWITCH	COMPUTED NUMBER OF TWITCHES PER INSPIRATION ASSUMING A COMMON FREQUENCY OF 10 PER SECOND	NUMBER OF ANIMALS OBSERVED
		seconds					
Horse.........	8	2.5	5–16	10	25	25	6
Dog..........	15	1.3	10–30	20	26	13	Many
Rabbit........	50	0.6	25–60	50	30	6	7
Rat...........	65	0.4	25–45	40	16	4	8
Mouse........	100	0.3	60–150	100	30	3	6

agreement with expected results (see table).   It is postulated that the number of twitches actually employed in the leading off units in a contraction forms the substructure upon which that contraction is built and that this substructure is suited to the purposes and to the control of that contraction.

The function and relative importance of the mechanisms of recruitment and frequency control.   To enquire more specifically into the function and relative importance of the factors of recruitment and frequency in the gradation of strength of muscular contraction, an artificial schema of multifiber twitching was constructed in which the frequency factor was omitted (see fig. 2).   The muscle units were arbitrarily scheduled to twitch at a uniform frequency of 7 per second, with intervening recruitment intervals of one forty-ninth of a second.   Each muscle unit is numbered in the order of its recruitment along the line A.F.   Units 1, 2, 3, 4, 5, 6, and 7 contract successively but never in unison.   Reinforcement is therefore wanting until coincidence of twitch 1 of unit 8 with twitch 2 of unit 1, doubles the intensity of contraction.   Serial coincidence of twitch 1 of units 9–14 with twitch 2 of units 2–7 maintains this doubled intensity.   Progressively

increasing coincidence increases the strength of contraction by 1 muscle unit per ¼ of a second.   At the end of 4 seconds the leading off unit will have twitched 28 times and that unit will have been supported by 27 additional units.

The maximum theoretical limit of gradation of muscular contraction by recruitment may be expressed by the fraction $X/1$ where X equals the total number of recruitable units in a muscle and 1 represents the mimimal recruitment, i.e., of one muscle unit.   The maximum limit of gradation of contraction by twitch frequency control is according to the direct measure of Adrian and Bronk (1928) significantly smaller, approximately a quadrupling of strength. The fraction $X/4$ would therefore represent the relative importance of recruitment and twitch frequency control.   Actually X must vary tremendously with size and structure running well into the thousands in some muscles.   In larger animals where the number of recruitable motor nerve cells and their corresponding muscle units is proportionately greater (see D'Arcy W. Thompson, *Growth and form*, 1917), recruitment as a power factor must play an almost exclusive rôle in the gradation of muscular contraction.

It is obvious that a shortening of recruiting intervals must result in a greater sum total of recruitment, and a finer gradation of contraction.   Were the mouse, e.g., to integrate its extremely short inspiratory contractions on the same fiber twitch frequency as that of the horse there could be only 3 twitches per inspiration in any single unit (see fig. 3).   Mechanical reinforcement of contraction would of necessity be small and gradation of contraction rough and jerky, a trebling of strength occurring in but 2 stages.   In the rabbit, strength of contraction would increase 7 fold, in 6 stages.   In the dog, the contraction would increase 13 fold in 12 stages and in the horse 25 fold in 24 stages.

Factors bearing on the mechanism and the site of recruitment and frequency control.   Though *frequency of muscle fiber twitch* may exercise small mechanical advantages, *frequency of nerve cell discharge* may nevertheless be a factor of major importance in the control of muscular contraction.   This hinges on the effective mechanisms of central summation of stimulation.   Witness the intensification of spinal reflexes by either a prolongation or an increased frequency of repetitive stimulation.   By analogy a discharging motor neuron, granting recurrent collateral and intermotor connections (Ramon y Cajal, 1909; Retzius and Linhossek—reference Ramon y Cajal; Gesell, 1940—figs. 41 and 42), could create a similar augmentation of recruitment and frequency of discharge in all interconnected motor neurons (see Gesell, Hansen and Worzniak, 1943).   Thus a prolonged series of discharges in a leading-off motor neuron, like a simple prolongation of sensory stimulation, and an increasing frequency of motor discharge, like a more frequent sensory stimulation, would favor an accumulative pooling of acetylcholine at interconnected motor neurons proportional to the sum total of nerve cell discharges.   Such pooling would automatically provide a *central* mechanism of progressive intensification of contraction.

Lightness of bombardment and absence of truly converging motor impulses in skeletal muscle on the other hand prevent similar integrations in the periphery.[3]

[3] Pleurisegmental innervation of muscle (Cattell, 1928, and Katz and Kuffler, 1941) can hardly be regarded as the equivalent of central convergence because 2 or 3 pleuriseg-

The neuron generally regarded as extremely reactive to the fluctuating electrical effects of temporal changes in bombardment, is now shown to possess synaptic potentials of a highly sluggish time course as compared with the motor and plate potentials of muscle (Eccles, J. P., 1943). "The latent period is about 5–7 times longer, the rising phase initially about 10 times slower, and the summit time and rate of decay 15–20 times slower." In the light of our "electrotonic" or "humoro electrical" concepts of physiological stimulation such gross differences in time course are suited to the well-known characteristics of nervous and muscular function. If a slow time course of synaptic potentials is synonymous with a slow time course of release and decay of acetylcholine, opportunities prevail for a gradual pooling and depooling of acetylcholine conforming with nicely graded summation (i.e., recruitment and acceleration of frequency of nerve cell discharge) and progressively diminishing after-discharge so common to nervous function. The rapid time course of motor end plate potentials (rapid release and decay of acetylcholine) on the contrary is correspondingly resistant to pooling.

Thus muscle and nerve would seem to differ in the nature of their stimulation. In muscle stimulation is electroclonic or pulsatile. Individual muscle fibers are stimulated once by a single, transient and private motor end plate potential. In nerve cells stimulation is electrotonic or non pulsatile and collective. Heterogeneous impulses impinging on the neuron by virtue of their force of numbers, their asynchronous order, and their sluggish synaptic potentials combine their overlapping effects into a collectively smoothed potential (Gesell, 1939, 1940; Gesell, Brassfield and Hansen, 1942). Direct experiments (Eccles, 1943) now show that prolonged and repetitive stimulation of preganglionic fibers sets up a steady, sustained, and greatly augmented synaptic potential. Such findings would seem to establish the existence of a continuing excitatory current as described in our "electrotonic" and "humoro electrical" theory in which it is proposed that the axon hillock (the point of emergence of the current) is fired rhythmically at a frequency proportional to current intensity.

Comparisons. From a comparative point of view, special mechanical advantages suitable to great size, mass, inertia and momentum conceivably accrue from slow rhythmical activity of muscle fibers and from prolonged muscular contractions. Slowness of pickup, such as occurs in the inspirations of a horse, should adapt mechanically to great mass and inertia. Great abundance of nerve cells and muscle units combined with ample time for contraction should assure the necessary power while still avoiding excessive acceleration. The mouse on the other hand would seem to operate on a somewhat different basis. In contrast to the horse, high frequency of twitch and smaller mass favor speedier pickup and nimbler gait, thus twitch frequency is adapted to the shortness and

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mental impulses arriving at a common muscle fiber produce *independent stimulation at each site of impingement.* Though mechanical summation occurs there is no evidence and no conceivable mechanism for the summation of such widely separated stimuli. An inherent rhythmic response to bombardment such as witnessed in the neuron is normally missing and only under conditions more comparable to nerve cell stimulation, produced by the protective action of eserine, can a release of acetylcholine at a motor end plate produce a rhythmic twitching.

lightness of its appendages. If diminutive animals are not to suffer unduly from the speed of their larger competitors and enemies, the shortness of their appendages needs compensation by a corresponding shortening of progression cycles. Similarly were the mouse to breathe as slowly as the horse, gross periodical chemical changes must undoubtedly occur in the course of each respiratory cycle. Correction of this defect by an increase of the volume of its lungs would obviously lead to other disadvantages.

Difference in motor nervous activity in large and small animals raises questions of interrelationships and of cause and effect. Are all functions of the brain in small animals geared to the same high intensity as that of motor integration? Are animals quick in their movements equally quick in other nervous integrations—sensory perceptions, associations, etc.? Are the relationships between metabolism and nervous function causal? Is the heightened basal metabolism of smaller animals a result of a heightened frequency of activity of its cellular units or conversely is the heightened frequency discharge of a nerve cell a result of its heightened metabolic rate due to surface volume relation? Is there a comparative functional measure of life span in such animals as the mouse and horse measurable in units more accurate than time, e.g., such activities as the number of breaths and nerve cell discharges per life?

SUMMARY

Contractions of the diaphragm of the mouse, rat, rabbit, dog and horse show characteristic electrical patterns of multifiber twitching.

This similarity of twitching indicates a common evolutionary adaptation for meeting analogous mechanical requirements (an increasing resistance to an increasing pulmonary inflation as inspiration progresses).

The adaptation consists of a progressive recruitment of activity of dormant reserve muscle units and an increasing frequency of twitch of the individual activated units.

This adjustment is a primitive mechanism for it is found in muscular contractions of insects, reptiles and birds as well as mammals.

All evidence indicates that recruitment of reserve muscle units is a far more important mechanism of gradation of strength of contraction than is adjustment of twitch frequency.

Respiratory and motor nerve cell rhythm were found to be higher in general in the smaller and nimbler animals.

As a consequence of this parallelism, the number of discharges of those motor neurons which lead inspiratory contractions tend to be approximately equal. This equality offers comparable facilities for the control of the frequency and recruitment factors and helps to explain similarity of inspiratory contractions. Thus the events transpiring in the prolonged inspiration of a horse are accelerated and compressed, without basic change, into the shortened inspiration of a mouse.

REFERENCES

GESELL, R., A. K. ATKINSON AND R. C. BROWN. This Journal 131: 659, 1941.
GESELL, R. Ergebn. der Physiol. 43: 477, 1940.

ADRIAN, E. D. AND D. W. BRONK. J. Physiol. **66:** 81, 1928.

BRONK, D. W. AND L. K. FERGUSON. This Journal **110:** 700, 1935.

LOOFBOURROW, G. M. AND R. GESELL. This Journal **133:** P365, 1941.

THOMPSON, D'A. W. Growth and form, Cambridge Univ. Press, 1917.

RAMON Y CAJAL. Histologie du Systeme Nerveux. Vol. 1: p. 753, Paris, A. Maloine, 1909.

RETZIUS AND LINHOSSEK. (Reference RAMON Y CAJAL).

CATTELL, McK. J. Physiol. **66:** 431, 1928.

KATZ, B. AND S. W. KUFFLER. J. Neurophysiol. **4:** 209, 1941.

GESELL, R., E. T. HANSEN AND J. J. WORZNIAK. This Journal **138:** 776, 1943.

ECCLES, J. C. J. Physiol. **101:** 465, 1943.

GESELL, R. Univ. Mich. Hosp. Bull. **5:** 12, 1939.

GESELL, R., C. R. BRASSFIELD AND E. T. HANSEN. Fed. Proc. **1:** 29, 1942.

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